New home, new life: The effect of shifts in the habitat choice of salamander larvae on population performance and their effect on pond invertebrate communities

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Erklärung des Promovenden

Die Übereinstimmung dieses Exemplars mit dem Original der Dissertation zum Thema:

„ New home, new life: The effect of shifts in the habitat choice of salamander larvae on population performance and their effect on pond invertebrate communities“

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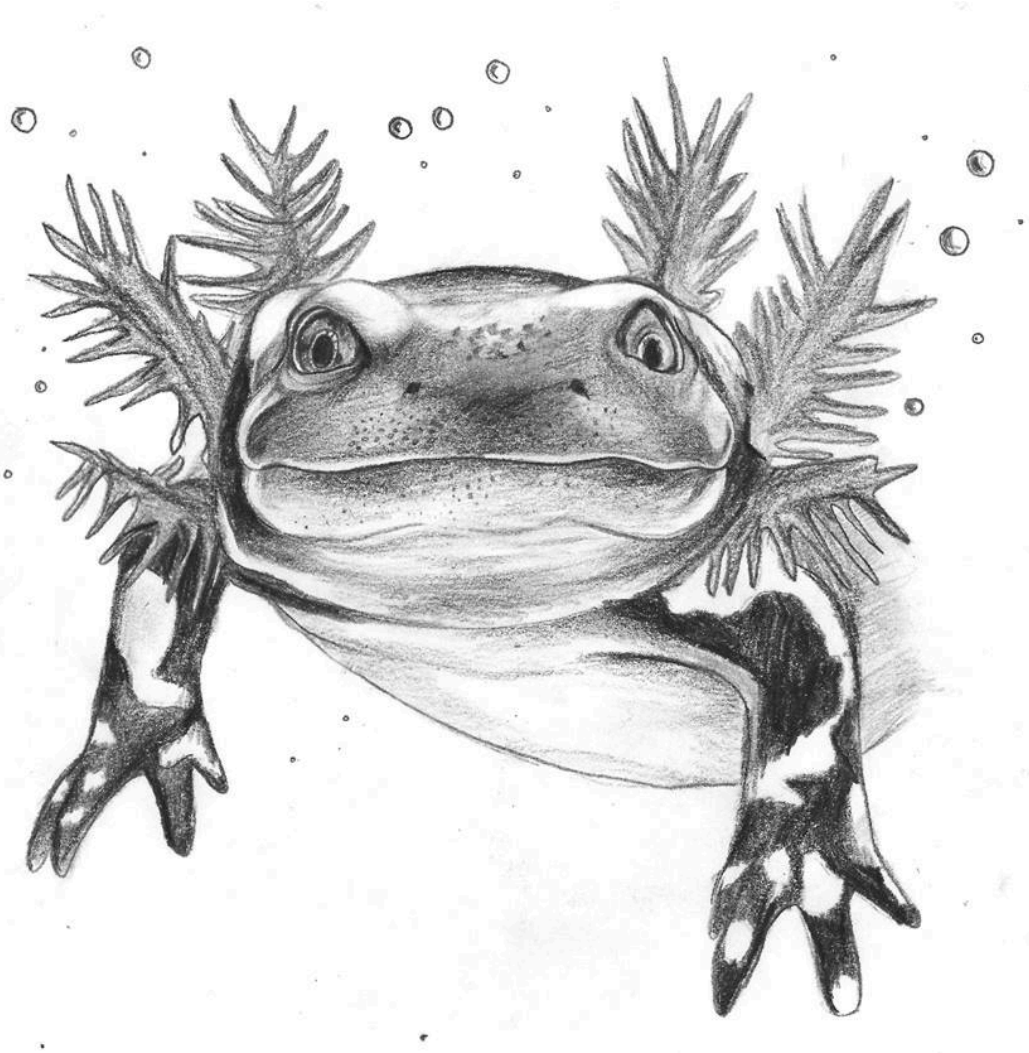

"*Of all venomous animals it is the salamander, that is the most dangerous; for while others attack individuals only [...] the salamander is able to destroy whole nations at once, unless they take the proper precautions against it.*"

–Pliny the Elder (ca. 77 A.D.)

Naturalis Historia

Cover picture created and kindly provided by Linda Wefers

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Zusammenfassung

Umweltveränderungen sind wichtige Faktoren, die Evolutionsprozesse einleiten können. Die durch sie bedingten Verschiebungen im Lebenzyklus oder Verhalten einer Art können in direkter Folge die Prozesse und Attribute ganzer Ökosysteme verändern. Ein Beispiel für eine rezente lokale Anpassung an einen neuen Lebensraum ist der Wechsel des Larvalhabitats des Westlichen Feuersalamanders (*Salamandra salamandra*) von Bächen in temporäre Standgewässer. Feuersalamanderlarven sind bedeutende Top- Prädatoren in fischfreien Kleingewässern und ihre Anwesenheit kann potentiell verschiedene Funktionen ihrer Ökosysteme beeinflussen. In dieser Studie wurde analysiert, wie die ökologische Performance von Feuersalamanderlarven in Standgewässern im Kottenforst bei Bonn (Deutschland) sich im Vergleich zu sympatrischen Bachpopulationen verändert. Desweiteren wurde bewertet, wie sich die Anwesenheit von Salamanderlarven in Teichen auf Schlüsselfunktionen im Ökosystem, wie Beutevorkommen und Diversität sowie aquatisch-terrestrische Kopplung, auswirkt. Um den Einfluss des Habitatshifts auf Ökosystemfunktionen beobachten und abschätzen zu können, wurden detaillierte Untersuchungen zur Phenologie und Populationsdynamik der Salamanderlarven mit Daten zur Zusammensetzung und Entwicklung der Makroinvertebratengemeinschaft, Mageninhaltsanalysen und experimenteller Manipulation von Teichen kombiniert. Im ersten Teil dieser Studie wurde die Auswirkung der Anwesenheit von Salamanderlarven in Teichen vor allem auf die aquatisch-terrestrische Kopplung analysiert. Es konnte gezeigt werden, dass es zu einem starken Anstieg an Import von terrestrischer Biomasse ins aquatische System kommt. Basierend auf der Hypothese, dass die massive Präsenz der Salamanderlarven einen direkten Einfluss auch auf die Makroinvertebratengemeinschaft haben,wurde dieser Einfluss im Weiteren experimentell getestet. Hier konnte jedoch gezeigt werden, dass die Salamander zwar einzelne Taxa stark beeinflussen, aber nur einen sehr eingeschränkten Effekt auf die Nahrungssnetzstruktur und Biodiversität der aquatischen Habitate haben. Trotz allem konnte bestätigt werden, dass der Stoffaustausch zwischen aquatischem und terrestrischem Lebensraum stark erhöht wird, was vor allem die hohen Positionen im Nahrungsnetz stabilisiert. Des Weiteren

wurde gezeigt, dass der Erfolg und das Verhalten der Salamanderlarven sehr starken zwischenjährlichen Schwankungen unterworfen sind, die vor allem mit den sehr unterschiedlichen Habitatsettings in verschiedenen Jahren verbunden sind. Hierin unterscheiden sich die Teichhabitate vor allem von den Bachhabitaten. . Schlussendlich konnte gezeigt werden, dass diese ökosystemaren Unterschiede und Restriktionen vermutlich auch für die genetische Aufspaltung der beiden Ökotypen von Bedeutung ist.

Abstract

Changes of habitats are amongst the main drivers of evolutionary processes. Corresponding shifts in the behaviour and life history traits of species might in turn also alter ecosystem attributes. The reproduction of Western European fire salamanders (*Salamandra salamandra*), in small pond habitats instead of first order streams, is one example of a recent local adaptation. Since fire salamander larvae are important top-predators in these fish free habitats, their presence likely changes various aspects of ecosystem functioning. Here, it was analysed how the ecological performance of salamander larvae in ponds in the Kottenforst in Western Germany changed in comparison to sympatric stream populations. Further, it was analysed how their presence in ponds influenced key ecosystem attributes such as prey density and diversity and aquatic-terrestrial linkage. To assess the impact of the life cycle shifts in salamanders on the pond functioning, detailed investigations of salamander larvae population dynamics, phenology, and macroinvertebrate community development in ponds were combined with experimental manipulations of the salamander presence. In the first part of this study, the impact of pond presence of fire salamanders in terms of ecosystem functioning focussing on aquatic terrestrial subsidy transfer was calculated. The study could show, that the adaptation of fire salamanders to breed in pools led to strong increases of animal-mediated import of terrestrial matter into the aquatic habitats. The hypothesis about the impact on macroinvertebrate communities derived from these calculations was then tested experimentally. It was shown, that presence of salamander larvae could influence some taxa of macroinvertebrates but they had only limited effects on the food web structure in their aquatic habitats. Yet, a high relevance of the subsidy exchange from aquatic to terrestrial and its high relevance for the predator persistence in the system could again be confirmed. Moreover, it was demonstrated, that the larval behaviour and performance could have a high inter-annual variability as a reaction to contrasting ecosystem constraints in comparison to the stream habitats. A fact that integrally separates the pond ecotype from stream ecotype conspecifics.

General Introduction

Amphibians and their larvae are often present in small aquatic habitats in enormous abundances (Regester et al. 2006; Gibbons et al. 2006). By this remarkably dominant presence, they can have important influence on the community and in turn ecosystem functioning. The majority of amphibian species displays a biphasic life cycle that has strong effects on reciprocal flows of residues between aquatic and terrestrial habitats by ovipositioning or birth and metamorphosis (Regester et al. 2006). Furthermore during their larval time amphibians can also act as important consumers in aquatic habitats. The larvae of anurans (i.e. frogs and toads) commonly consume detritus and biofilms (Altig et al. 2007) and can thus efficiently utilise primary resources. The larvae of urodelans (newts and salamanders) on the other hand are predators. Those play a role as top predators especially in systems where fish are absent. As such, they can integrally influence the structure of the food web structure (Holomuzki et al. 1994; Blaustein et al. 1996) by controlling invertebrate biodiversity, abundance and productivity. This impact on prey communities in turn can influence the transfer of subsidies by reduction of insects that also plays a role in matter transfer.

The diverse ecosystem connections exerted by amphibians make this taxonomic group a valuable indicator for ecosystem functionality and condition (Ryan 1986). Their role as ecosystem alert system is of increasing importance as amphibians are currently worldwide amongst the most quickly declining and threatened classes of vertebrates (Whiles et al. 2006). Infectious diseases such as Chytridiomycosis, increased degradation and fragmentation of their habitats (Marsh and Trenham 2001; Skelly et al. 2003) as well as global climate change (Pounds 2001; Pounds et al. 2006) are responsible for global population declines of amphibian species (Pounds et al. 2006; Sodhi et al. 2008). While this amphibian crisis has been gaining increasing awareness in the current years, still very little is known on the importance of amphibians for the mentioned habitat functions. This is especially true taking into account that a lot of amphibian species can react flexibly to ecosystem changes (Cushman 2006) and adapt to new habitats, which in turn will also alter their ecosystem functions. Habitat shifts are usually connected with new environmental restrictions and selective pressures that require new adaptations and can increase genetic diversity and drive speciation processes (Martin and Pfennig 2009).

The present thesis aims at shedding some light into the ecological role and adaptive traits of one species of amphibian, the western fire salamander (*Salamandra salamandra LINNEUS 1758*) and relate its ecosystem functions to ongoing evolutionary shifts. The fire salamanders (a group of species within the genus *Salamandr*a) are wide spread throughout Europe. Fire salamanders are the only Urodelans that give birth to fully developed larvae into the aquatic habitat while the adults are exclusively terrestrial (Thiesmeier 2004). Their larvae can be found in different fish free aquatic habitats such as first order streams and ephemeral ponds in which they often comprise the largest predators (Thiesmeier 1992; Blaustein et al. 1996). In Western Europe *Salamandra salamandra* is a typical and common inhabitant of old broad leaf forests in mountainous areas. Here most populations deposit their larvae in headwater streams but some have adapted to use other waterbodies such as drainage ditches, wheel ruts and natural water-filled depressions. These ephemeral ponds in temperate regions are a very special kind of aquatic habitat as a result of their small size and limited time of existence. In contrast to first order streams, they often are highly instable habitats in terms of temperature fluctuations, oxygen content and chemical parameters (Wellborn et al. 1996; Williams 1996; Brooks 2000). The unpredictable conditions require special strategies for pond organisms that often result in a flexible phenology (Williams 1987). On the other hand, ephemeral ponds can receive high amounts of subsidies, that can drive community dynamics and food web stability (Rubbo et al. 2006). In fact the pond and the stream breeding ecotype of the western fire salamander could be shown to be genetically distinct. This separation process could be demonstrated the result of a recent local adaptation process (Weitere et al. 2004; Steinfartz et al. 2007b).

This study aims at analysing the effects of pond reproduction behaviour on the pond breeding invertebrate communities and corresponding ecosystem processes. It especially focuses on the role of fire salamander for aquatic terrestrial subsidy exchanges and the trophic control of food web structure in ponds. Furthermore, it will characterise the phenological adaptation of pond reproducing fire salamanders under the instable conditions in pond habitats, which is the main difference to the original stream environment of the species. Thereby it will detect factors potentially driving the evolutionary shift of habitat selection from stream to pond habitats in this species.

The thesis will present these topics in four chapters:

Chapter 1 will introduce into ecological functions fire salamander larvae can exert in pond environments. Based on indirect calculations we provide an estimate of animal-mediated transfer of matter from forest to the aquatic system. Here data based on biomass of the present pond fauna as well as on the analysis of stomach content data, growth rates and population dynamics of the salamander larvae in pond habitats are used. The chapter shows, that due to the great amount of salamander larvae in ponds, the trophic pyramid is inverted resulting in strong top down regulation of invertebrate communities. Further it is calculated that this greatly increases subsidy transfer into the aquatic system both directly (as through birth and metamorphosis) and indirectly (through consumptive effects). Both calculations will be taken into account again in chapter 3.

Chapter 2 deals with the inter-annual variability of pond habitats, which is of special relevance in the pond habitats. The high inter-annual variation and instability of environmental settings should influence the phenology and life history strategies of fire salamander larvae in ponds. It is analysed here, how the framework of temperature and rainfall influences developmental patterns and behaviour of the larvae in both direct and indirect (as mediated by prey) ways. It analyses also which drivers can provide flexible life cycle solutions to cope in a highly variable environment.

Chapter 3 tests the role of fire salamander larvae as top predators in ephemeral ponds. In this chapter experimental data from pond manipulations are provided to validate the in chapter 1 indirectly calculated impact on prey species diversity community composition and ecosystem functioning. Also it determines how the community of the small ephemeral ponds can sustain high predator densities by terrestrial subsidization of the food web.

Chapter 4 compares the ecological performance and success of larvae of pond and stream reproducing salamanders in relation to biotic and abiotic environmental factors. It finds differences in larviposition timing and growth patterns as well as resource usage. These are related to environmental constrains such as temperature, hydrological regime and food availability. Here it is especially considered, which selective drivers act on the larval populations and could drive the genetic separation of the two ecotypes.

1. Chapter

Linking the evolution of habitat choice to ecosystem functioning: Direct and indirect effects of pond-reproducing fire salamanders on aquatic - terrestrial subsidies

1.1 Introduction

The across-habitat exchange of nutrients, detritus, prey and consumers can play an important role in the functioning of ecosystems, and is gaining increasing attention in functional ecology as well as ecosystem management (Polis et al. 1997; Knight et al. 2005; Leroux and Loreau 2008). Spatial linkages occur due to abiotic factors distributing nutrients or detritus between ecosystems, or due to active exchange of living organisms between two types of habitat (Polis et al. 1997). Examples for the latter include emergent aquatic insects, which can be consumed by riparian arthropods (Paetzold et al. 2005), birds (Murakami and Nakano 2001) and lizards (Bastow et al. 2002) foraging in riparian environments or terrestrial insects consumed by fish (Allan et al. 2003; Kawaguchi et al. 2003).

Especially, animals with complex life cycles involving one or more changes in habitat can have strong effects on reciprocal resource fluxes between adjacent ecosystems (Baxter et al. 2005; Schreiber and Rudolf 2008; Paetzold et al. 2011). This aspect is particularly true for most amphibian and many insect species, which have aquatic larval phases and a terrestrial adult phase. Such emergent animals can significantly subsidise aquatic (Regester et al. 2008) and terrestrial (Baxter et al. 2005) consumer communities. Regarding the effects of amphibians on aquatic ecosystems, different trophic guilds exist among the larvae. Most tadpoles of frogs and toads are grazers or collectors that feed on lower trophic levels, particularly planktonic and periphytic algae and bacteria (Ranvestel et al. 2004; Altig et al. 2007; Verburg et al. 2007). Accordingly, this low position in the consumer food web appears perfect for an efficient exploitation of the primary production.

This situation is not the case in the larvae of tailed amphibians, i.e. newt and salamander larvae, which are predators and can be found in significant quantities in the aquatic habitat. After hatching or birth, they can exert strong top-down pressure on the aquatic

invertebrates and alter the aquatic food web structure (Holomuzki et al. 1994; Blaustein et al. 1996; Walls and Williams 2001). Thus, they have the potential to alter the flux between aquatic and terrestrial systems by (i) feeding on emergent amphibians and insects in ponds, therefore preventing their export to the terrestrial environment (Holomuzki et al. 1994; Regester et al. 2008), and (ii) feeding on terrestrial animals which fall onto the water's surface (Denoel and Joly 2001; Denoël and Denoel 2004) (Fig. 1.1). The magnitude of such indirect effects of salamander larvae on the aquatic terrestrial linkage is yet still unknown.

The fire salamander (*Salamandra salamandra*) is a dominant and widely distributed species in central Europe. The adults inhabit old broadleaf forests and typically deposit their larvae into first order streams. In this fish-free habitat, they are the top vertebrate predators (Thiesmeier 2004). As members of the true salamanders, the different species of fire salamanders (see Steinfartz et al. 2007 for systematic and phylogenetic details) are the only extant tailed amphibians (Urodela) that give birth to fully developed larvae (called larvipary; see Greven 1998), which are deposited into aquatic habitats. In contrast to pond-breeding central European newts, the larva of the fire salamander is distinctly larger; its aquatic energy uptake during the larval period is thus relatively high. Considering this high energetic demand at a top-position within the aquatic food web, the risk of resource overexploitation exists, particularly in small habitats. The stream reproductive mode should be beneficial for the survival of the larvae, as drift is used as a strategy to regulate population densities, if crowding of larvae leads to local starvation (Thiesmeier 1992). Such behaviour prevents extremely high densities and thus reduces the magnitude of intraspecific competition for food. However, some populations of fire salamanders reproduce partially or even exclusively in standing waters (Blaustein et al. 1996; Thiesmeier 2004; Manenti and Ficetola 2011), in which a compensatory drift is impossible.

We have studied a population of fire salamanders in North Rhine-Westphalia (Germany), in which pond-reproduction has evolved as a habitat specific adaptation following the last glaciation (Weitere et al. 2004), resulting in genetic differentiation between pond- and stream-reproducing salamanders within the same forest population (Steinfartz et al. 2007b). The pond type is characterised by relatively large larvae at the time of deposition and a reduced threshold size at metamorphosis (Weitere et al. 2004).

Nevertheless, the energy demand in the pond is high, and top-down effects on the pond-breeding invertebrates are thus probably large.

Here, the effects of this habitat specific adaption on the subsidy exchange between aquatic and terrestrial ecosystems were analysed. Specifically, it was hypothesised that the adaptation of salamanders to pond reproduction increases the animal-mediated transfer of matter from forest to pond. I paid special attention to both the direct (deposition, metamorphosis) and indirect (predation on terrestrial and emergent invertebrates) role of these amphibians in the trophic coupling of their pond habitats to the terrestrial environment (see Fig. 1. 1).

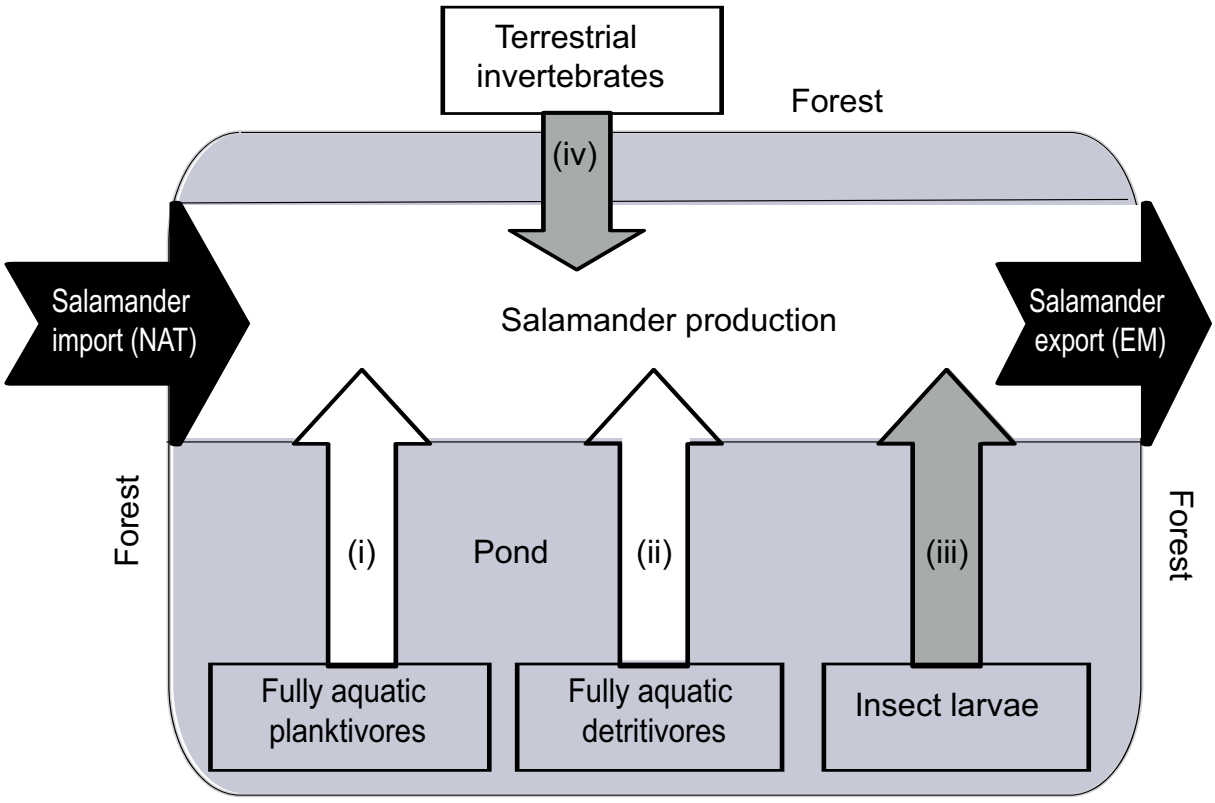

Fig. 1.1: Pathways of salamander-mediated biomass flux in pond food webs and between pond and forest. The arrows (NAT) and (EM) represent direct salamander-mediated biomass flux between pool and forest (natality and emergence). Boxes represent standing crops of food organisms, arrows i-iv represent consumption by salamander larvae. Light grey arrows indicates that this consumption results in indirect salamander-mediated flux between the habitats, whereas white arrows indicate neutral interactions with respect to aquatic/terrestrial coupling.

1.2 Material and Methods

Study site and salamander population

The investigated breeding ponds were located in the Königsdorfer Wald near Cologne, Germany (N 50°56.425´ E 006°44.132´). The area has a size of approximately 3.5 km^2 and lies isolated, surrounded by settlements and a highway to the east and south and the brown-coal spoil tip Glessener Höhe to the north. It is an old broadleaf forest with European beech (*Fagus sylvestris*) and sessile oak (*Quercus petraea*) as the predominant tree species, and represents a typical terrestrial habitat of the fire salamander. The soil of the forest is stagnosolic, i.e. poorly drained, and hence periodically allows the formation of various ponds and puddles during times of high rainfall. However, the salamanders' breeding behaviour is atypical here in that most of the individuals (>80% of the population) breed in ponds instead of in first-order streams. This behaviour was the result of a recent (postglacial) adaption (Weitere et al. 2004, see above).

All data used in this study were gathered between February and October 1996; parts of the dataset have already been published in Weitere et al. (2004), focusing on the ongoing differentiation process of ecologically differently adapted fire salamander types of that region. In this present analysis of the dataset, all water bodies within the forest that contained salamander larvae and have hydroperiods sufficiently long enough to facilitate significant larval development were considered Investigated ponds included bomb craters (as indicated by prefix "B", see Table 1.1), roadside drainage ponds ("G"), wheel ruts ("W"), a rainwater storage basin ("R") and the artificial spring ponds of the only stream site ("S"). The ponds R1, G2 and G3 were ephemeral in 1996, whereas all other ponds under investigation retained water at least until October (Table 1.1).

The investigated ponds varied in several aspects of hydrology and abiotic conditions. The water level was measured at each sampling date and surface area was measured over the complete breeding period (Table 1.1). The temperature was monitored in each pond with the help of a maximum-minimum thermometer at a water depth of 15 cm, if possible. Oxygen content (sampling probe Oxi 191, WTW) and ammonium content (Ammonium Spectroquant 14752) of the breeding waters were also measured regularly. Oxygen saturation in the open water column was below 50% for most ponds throughout the day. The bottom of the ponds B1, B2, G1, G2, G3, W1, and W2 were covered with thick layers of leaf litter and other terrestrial detritus, and were characterised by turbid water. In G1 and W1, the deep detritus layers and ongoing decomposition processes led

to oxygen depletion, particularly during the night. High temperatures (>30°C), low oxygen saturation (<10%) and pond drying were associated with salamander mortality. Here we selected six ponds, which represent typical salamander habitats with respect to biotic and abiotic conditions, for detailed estimations and calculations.

Characteristics of the reproduction habitat and food organisms

Macrozoobenthos and zooplankton was sampled three times within the larval period in the selected ponds (exception: two samples in R1 due to pond drying in June) to compare the ponds' biological properties and to estimate the standing crop of prey available to the salamander larvae. Three replicate samples were considered for each pond and sampling date. The invertebrates were classified as to whether or not they were potential salamander food, i.e. either as edible or inedible according to their size. The classification was conducted on the basis of stomach content analyses of the salamander larvae (see below). Only the edible portions of the pond fauna was sampled and considered in the following. For a detailed description of the sampling methods used to obtain the food organisms, see Weitere et al. (2004). The body size of all sampled individuals was measured and the dry mass (DM) was estimated for all organisms using size to body mass correlations, as according to Benke et al. (1999) for aquatic insect larvae, Rogers et al. (1976; 1977) for insect imagines and Dumont et al. (1975) for planktonic crustaceans.

Quantification of direct salamander-mediated material flux: natality, emergence and biomass

Unlike all other central European amphibians, the fire salamander is larviparous. Larval deposition usually occurs discontinuously during rainy nights in early spring (Thiesmeier 2004; Weitere et al. 2004). Larval import was estimated by quantifying larval densities immediately after such deposition events (see next section for methods). The total biomass flux from forest to pond through larval deposition was estimated from the larval number multiplied by the average body weight at birth according to Weitere et al. (2004). Salamander wet mass was converted to dry mass as according to (Crump 1979). For an estimation of the salamander-mediated biomass export, the number and body masses of emerging salamander larvae were quantified. For this purpose, sampling of the salamander larvae was intensified (every three days) during the time of potential

metamorphosis. All salamander larvae showing advanced signs of metamorphosis, i.e. particularly significant fin and gill reduction, were sampled. As metamorphosing salamanders cease feeding in this late stage of metamorphosis (Gasche 1939), they were kept in the laboratory at 20°C until metamorphosis was complete, and their weight at metamorphosis was immediately measured. The biomass exported by salamanders was considered as the sum of all individual biomasses at metamorphosis for each pond site.

Quantification of indirect, salamander-mediated matter flux, larval production and food requirements

We estimated larval densities of all ponds by catch-per unit effort adjusted for each pond by a capture-recapture method (Youngs and Robson 1978).

As many larvae as possible were caught within ten minutes using a standardised triangular amphibian scoop net with 50 cm width at the base and a mesh size of 0.5 cm (Schlüpmann et al. 1995). For the capture-recapture method, all larvae were marked by digit amputation and released back into the pond immediately after marking. Recapturing of larvae was conducted the following day. The total number of individuals (N) in a single pond was calculated using the number of marked larvae (m), the number of recaptured larvae (c) and the number of marked larvae among the recaptured larvae (r), with the following equation following Youngs and Robson (1978):

$$N = \frac{(m x c)}{r} \quad (1)$$

This detailed estimate was performed every 10-20 days for each pond. In between (approx. every seven days), the number of larvae caught within ten minutes was used to check for the occurrence of larger population fluctuations. By taking biomass import, biomass export, larval densities and individual larval growth into account, the total salamander larval production in each pond was estimated. Weight and length development of the larvae in the six ponds was measured throughout the breeding season by considering average growth within cohorts of marked larvae. Early within the breeding season, cohort growth was measured every ten days from March until May. For this purpose, 30 individuals per location were weighed and marked according to their size cohorts and released to monitor cohort growth. When the population had decreased below 30 individuals per pond (which occurred by the end of June), all remaining larvae were marked and reweighed every two weeks, yielding individual growth data. We calculated mean growth rates from these data to compare the biomass increase of larvae

in the different ponds. Growth rates (g, d^{-1}) for each sampling interval was calculated using the following equation:

$$g = \frac{\ln(W_{t+1}) - \ln(W_t)}{t} \quad (2)$$

In this equation (2), W_t is the mean larval biomass at the start, W_{t+1} the biomass on the following sampling date, and t indicates the time period (d) between the two sampling dates. The mean body mass production (P, mg DM d^{-1} m^{-2}) of the salamander larvae was calculated from the mean growth rate for each sampling interval (g) multiplied by population density (D, ind. m^{-2}) and the mean body mass of all sampled individuals (B, mg DM $ind.^{-1}$).

$$P = g \times D \times B \quad (3)$$

The sum of complete daily production (P, mg DM m^{-2} d^{-1}) during the entire investigation allows an estimation of the seasonal production $P_{(season)}$. This seasonal production was used, to estimate the minimal potential food requirement of the larvae population per m^2 (REQ, mg DM yr^{-1} m^{-2}) throughout the season. For this purpose a gross growth efficiency (GGE) of 0.34 for benthic prey and 0.31 for zooplankton crustaceans, as developed for salamander larvae by Regester et al. (2008) was used. These values are based on an assimilation efficiency of 0.64 for chironomids and 0.62 for zooplankton crustaceans measured by Regester et al. (2008) multiplied with a net assimilation efficiency of 0.5 (Burton and Likens 1975).

$$REQ = P_{(season)} \times GGE^{-1} \quad (4)$$

I am aware of the fact that the GGE decreases with decreasing food supply and also depends on temperature (Straile 1997). Particularly low food conditions can occur in the ponds investigated here, and the GGE might thus have dropped significantly below 0.3 in our present study. Thus, our approach of using a constant GGE can lead to an underestimation of the food requirement (REQ); I therefore consider the value as minimal food requirement. However, no salamander growth occurred in one pond (G1). In this case REQ could not be estimated.

Food spectrum analysis of salamander larvae and budget of salamander-mediated aquatic- terrestrial fluxes

To estimate the impact of salamander predation on the different functional groups of the aquatic invertebrates, the diets of the salamander larvae was examined from stomach content analyses. For this purpose, a total of 78 larvae from the ten different ponds were preserved in 4% formaldehyde solution immediately after being caught. All food items in the stomach were classified and measured. The body length of all organisms was measured, and the body mass was calculated using length dry weight correlations as described above. We classified the food organisms into functional groups according to their food preferences and life history traits (Fig.1.1): The groups were (i) fully aquatic planktivores feeding mainly on phytoplankton, bacteria and microfauna (e.g. planktonic cladocerans, copepods and benthic bivalves of the genus *Pisidium spp.*), (ii) fully aquatic detritivores which decompose allochthonous leaf litter (e.g. *Asellus aquaticus*, oligochaetes), (iii) insect larvae (with complex life cycles) and (iv) terrestrial invertebrates. The required food biomass REQ (see equation 4) multiplied by the percentage by mass of the specific functional group in the stomach contents (T) results in a value, which reflects the consumption of each functional group (C_x, mg DM yr^{-1} m^{-2}):

$$C_x = REQ \times T \quad (5)$$

Regarding the overall biomass fluxes between ponds and forest, direct biomass input by salamander natality (NAT), the consumption of insect larvae with complex life cycles (functional group iii; see Fig. 1.1) and consumed terrestrial invertebrates (functional group iv) were considered as flux into the aquatic system or the prevention/reduction of export (+). Biomass output by salamander emergence (EM) is considered as a flux into the terrestrial system (-) (see Fig. 1.1). The salamander-mediated flux through the ponds is therefore expressed by:

$$FLUX = NAT + C_{iii} + C_{iv} - EM \quad (6)$$

Positive FLUX values express an overall flow of biomass towards the aquatic habitat, negative values show a flow of biomass from the aquatic to the terrestrial system.

1.3 Results

Direct salamander larvae-mediated biomass flux

In general, the initial biomass import to the ponds by salamander natality was large compared to the biomass of food organisms available (Fig. 1.2). However, this proportion differed greatly among ponds. While the initial salamander biomass in the pond with highest salamander density (G1) was 23.9 times higher than the mean biomass of prey organisms, the salamander biomass in the pool with lowest salamander density (R1) was 11.3 times lower than the available biomass of prey organisms. In 1996 the first larvae were deposited on February 25th and major peaks occurred in March and April. Deposition of larvae was completed in all ponds by the end of May, resulting in larval densities of between 4 (R1) and 104 individuals m^{-2} (G1). The mean weight of the newly born larvae was 0.18 g ± 0.04 SD fresh weight (Weitere et al. 2004), corresponding to a dry mass of 0.03 g ± 0.01 SD. The primary biomass input by depositing females ranged from 0.07 g DM m^{-2} (R1) to 2.86 g DM m^{-2} (G1) (Table 1.2).

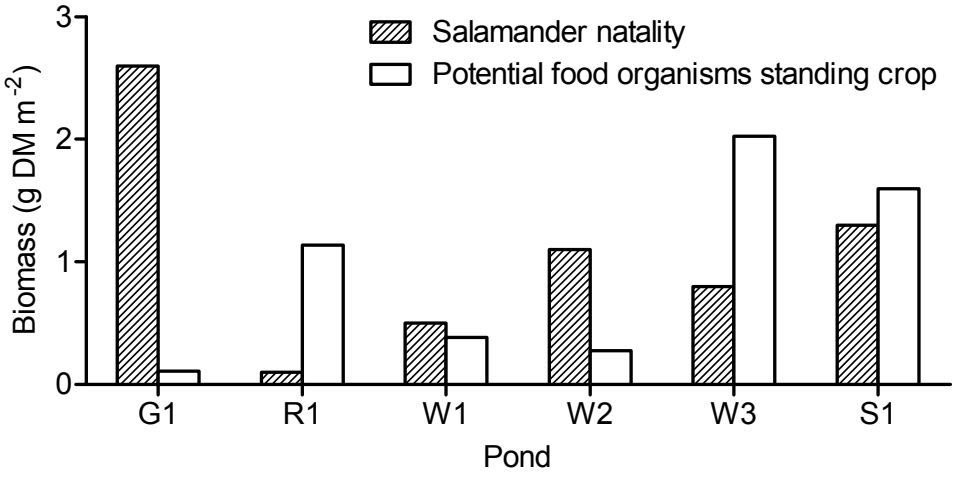

Fig. 1.2: Biomass of salamander natality as deposited by female fire salamanders (*Salamandra salamandra*) versus biomass of potential food organisms (grey bars; sample mean) in six ponds in central Europe.

Throughout the year, the biomass production of salamander larvae ranged from 0.01 g DM m^{-2} yr^{-1} in R1 to 0.13 g DM m^{-2} yr^{-1} in W3 (Table 1.2). Whereas ponds with high macrozoobenthos crops also had a high production of salamander biomass, those with

extremely low densities of macrozoobenthos (particularly G1) showed no significant production of salamander biomass. Total extinction of larvae occurred in G1, associated with decomposition-related oxygen depletion, and in R1, associated with high temperatures in June before metamorphosis could be completed (Table 1.1). The remaining larvae in the other ponds continued to emerge until the end of September, and represented the seasonal exports to the surrounding forest. Out of a total of 4160 larvae deposited in the ponds, 165 individuals reached metamorphosis, which represents a 4% emergence success of the initially deposited larvae. Average individual larval biomass at metamorphosis for all ponds was 0.11 g ± 0.02 SD dry mass (0.77 g ± 0.18 SD fresh mass). The amount of biomass that was imported into the ponds in spring exceeded the amount of biomass exported through metamorphosis in all ponds except W2 (Table 1.2). In the ponds where mass mortality occurred (G1 and R1), the entire salamander biomass remained in the aquatic system. In the remaining ponds, the direct flux of salamander biomass into the aquatic habitat ranged between -0.14 (W2) and 1.20 g DM m^{-2} yr^{-1} (S1).

Table 1.1: Abiotic conditions in the forest pools: The maximal depth and the pool area correspond to periods of mean water load. Critical factors which coincided with mass mortalities of the larvae are marked by bold printing, i.e. temperatures above 30°C and oxygen levels below 10% relative saturation (with the exception of the cold, groundwater-fed pool S2 in which no increased mortality occurred). The ranges of oxygen concentrations are given for relative saturation (%) and absolute concentrations (mg l^{-1}). The pools S1, G1, R1, W1, W2 and W3 (grey shading) were selected for detailed investigations.

Pond	Max. depth (cm)	Area (m^2)	Pool type	Hydroperiod (in 1996)	Shading (%)	Max. temperature during larval period (°C)	O_2 (%) and (mg l^{-1})	NH_4^+ (mg l^{-1})
S1	13	4.5	spring pot	permanent	50	11.5 (groundwater influenced)	40-47 (4.6-5.6)	Maximal 0.03
S2	40	3	groundwater storage basin	permanent	100	14.5 (groundwater influenced)	4 – 48 (0.4-5.8)	Maximal 0.13
B1	55	12	bomb crater	permanent	80	17.5	**8-39 (0.8-4.4)**	**8.8-13.1**
B2	30	15	bomb crater	permanent	95	17.5	10-44 (0.9-4.9)	4.4-7.1
G1	30	8	roadside drainage ditch	permanent	70	20.5	**7-51 (0.7-5.8)**	**1.2-9.1**
G2	30	52	roadside drainage ditch	Until mid June	50	**31.0**	61-184 (6.3 – 15.7)	0.03-0.09
G3	30	26	roadside drainage ditch	Until mid June	0	**31.0**	70 – 305 (7.1 – 35.1)	0.05-0.10
R1	35	72	rainwater storage basin	End of April and Mai – mid June	10	**31.0**	**5 – 170 (0.5 – 13.5)**	0.3 – 1.4
W1	30	13	wheel rut	permanent	80	20.5	**6 – 50 (0.6 – 5.1)**	**1.2 – 5.3**
W2	20	5	wheel rut	permanent	60	20.5	19-46 (1.9-4.8)	5.5-12.4
W3	20	7	wheel rut	permanent	60	19.0	17-54 (1.7-5.7)	4.2-6.4
others	No abiotic factors measured in pools which dried within 1 month after larval hatching							

Indirect biomass flux to ponds by salamander predation effects

Standing crops of macrozoobenthos and zooplankton available to salamander consumption varied among the ponds (Fig. 1.2). The highest standing crops of macroinvertebrates were recorded in W3 with an average crop of 2.00 g DM m^{-2}; the lowest crops were found in G1 with as little as 0.10 g DM m^{-2} (Fig. 1.2). Insect larvae especially chironomids were amongst the predominant taxa in most ponds. (Fig. 1.3A).

Overall, the salamander larvae consumed between 0.04 (R1) and 0.41 g DM m^{-2} yr^{-1} (W3) of food biomass (sum of all taxon specific consumption) until metamorphosis was completed. The salamander larvae fed on a wide variety of food taxa, but insect larvae were the preferred prey items found in the stomach content samples. Chironomid larvae accounted for 45% of all stomach contents, and insect larvae altogether accounted for 67% of all stomach content biomass (Fig. 1.3B). Between 0.01 and 0.37 g DM m^{-2} of purely insect biomass was reduced in the ponds per year due to salamander consumption (Table 1.2). Pelagic food organisms (cladocerans and copepods) comprised only a small fraction of the food spectrum of all larvae. Furthermore, the gut samples contained terrestrial invertebrates in all ponds except G1. Insects that fell on the water surface were often ingested and could account for as much as 75% of the consumed biomass in pond W1 (Fig. 1.3B).

Both the direct salamander biomass flux and the indirect pathways created a net flow of biomass into the aquatic habitats. The overall salamander-mediated flux (direct and indirect) between aquatic and terrestrial habitat thus resulted in a net import of biomass into in the aquatic systems of between 0.17 g DM m^{-2} yr^{-1} for the habitat with lowest biomass flux (W2) and more than 2.86 g DM m^{-2} yr^{-1} for G1. The latter comprises a minimal estimation as the actual flux could not be calculated (Table 1.2) because no growth was detectable under the extreme conditions in G1.

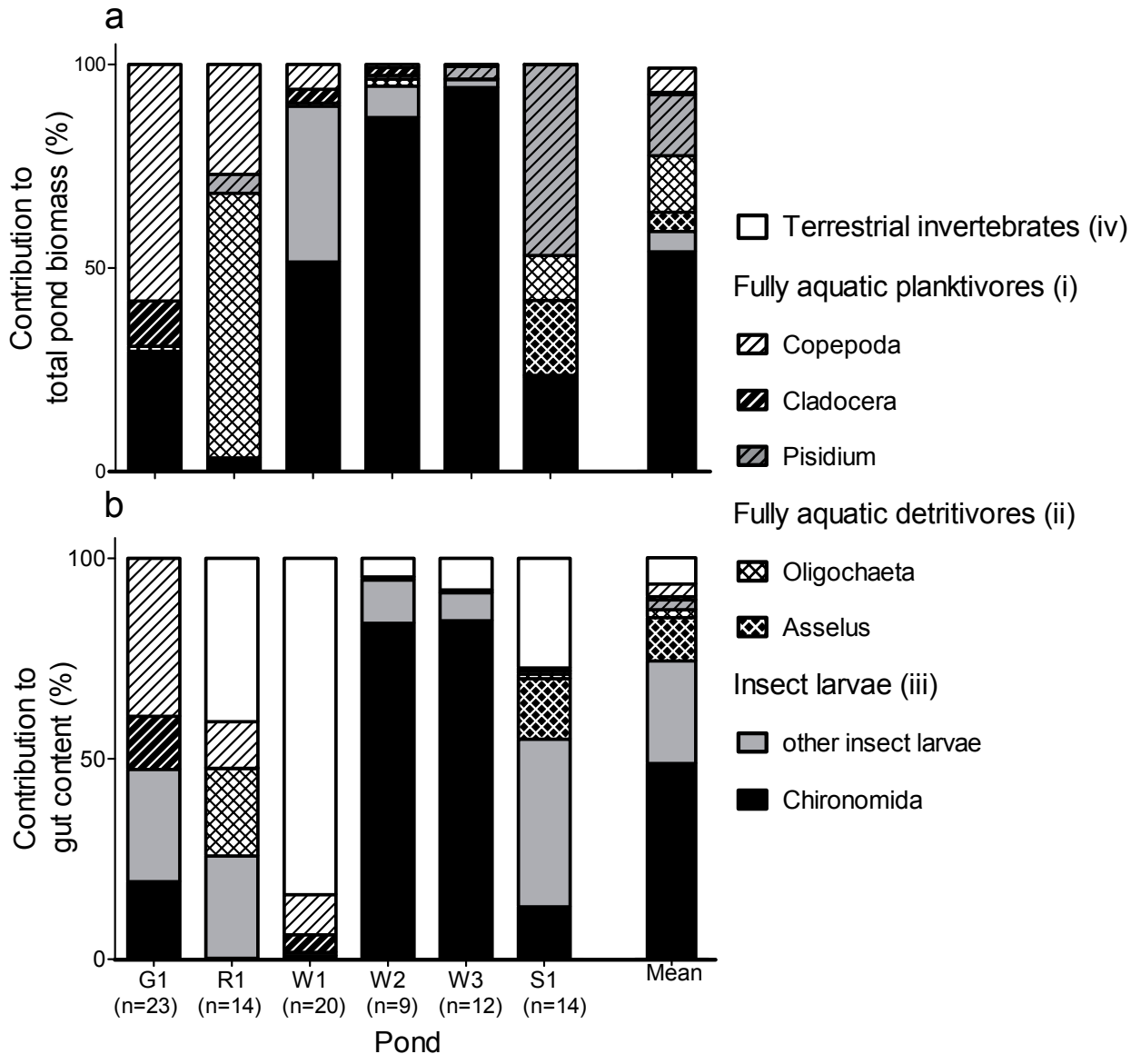

Fig 1.3. A) Edible pond fauna: taxonomic composition of potential salamander food organisms as percent of total standing crop biomass in six ponds (mean of all sampling dates per site). B) Prey selection: taxonomic composition of invertebrate food in salamander larvae stomach contents (n = number of sampled larvae). All food organisms are divided into functional groups (i-iv) defined in Fig. 1.1.

Table 1.2: Estimation of the direct and indirect salamander-mediated biomass flux between pool and forest. All values are given as fluxes (g DM m^{-2} yr^{-1}) represented by arrows (NAT, EM; i-iv) in Fig. 1.1 Numerical operators represent: (+) net import to pool, (-) net export from pool and (0) neutral biomass flux.

Pool	**NAT (+)** Salamander import	Salamander production	ii (0) Consumed fully aqu. detritivores	i (0) Consumed fully aqu. planktivores	iii (+) Consumed aqu. insect larvae	iv (+) Consumed terr. invertebrates	**EM (-)** Salamander export	**(=)** Total flux to pool
G1	2.86	0	0	n. e.*	n. e.*	0	0	>2.86
R1	0.07	0.01	0.01	<0.01	0.01	0.01	0	0.09
W1	0.51	0.03	0	0.01	0.01	0.06	0.10	0.48
W2	1.16	0.1	0	<0.01	0.29	0.01	1.30	0.17
W3	0.83	0.13	0	<0.01	0.37	0.03	0.52	0.71
S1	1.38	0.04	0.03	<0.01	0.08	0.04	0.18	1.32

*n.e: In G1 an estimation of the consumption was not possible since the individual larvae showed no net growth

1.4 Discussion

Previous studies have demonstrated that organism-mediated matter fluxes between physical boundaries of different habitats and can have profound effects on ecosystem attributes (Polis et al. 1997; Baxter et al. 2005; Post et al. 2007). Here we link this important function to evolutionary adaptations, demonstrating that changes in the breeding behaviour of a single species can strongly alter the dynamics of aquatic-terrestrial linkages. Nevertheless, the harsh conditions in the new breeding habitat can lead to mass mortalities, again altering the across-habitat flux. Moreover, by considering predators instead of herbivores or detritivores, it could be shown, that the indirect effects on subsidy transfer can even exceed its direct transfer of biomass.

Recent habitat adaptation alters ecosystem coupling

Evolutionary adaption can have major effects on ecosystem attributes such as food web structure, detritus decomposition, primary productivity and thus nutrient flux, as demonstrated for different fish species within aquatic habitats, such as for sticklebacks (Harmon et al. 2009) and guppies (Bassar et al. 2010; Bassar et al. 2012). Here we demonstrate that adaptive traits in an amphibian species can have significant cross-ecosystem effects by influencing subsidy exchange between two habitat types. By the evolution of the pond-reproducing type of the central European fires salamander (Weitere et al. 2004; Steinfartz et al. 2007b), both birth and metamorphosis of the larvae

result in new direct fluxes between the ponds and the terrestrial environment. Seale (1980) and Regester et al. (2006) demonstrated amphibian-mediated biomass imports of between 1.4 - 9.0 g DM m^{-2} to other pond systems, thus at levels similar to those observed in our study. Nevertheless, the amounts indentified in previous studies also included masses of egg-jelly and infertile eggs, which exclusively fuel the decomposition pathway and increase nutrient availability instead of developing into active predators, which alter the aquatic food web structure.

Pond-breeding urodelans such as newts (e.g. *Triturus* and *Lissotriton* species in central Europe) are also strictly oviparous and deposit eggs from which larvae hatch. Their complete development thus takes place in the ponds and, consequently, the newt density is regulated by the carrying capacity of the aquatic habitat. In contrast, fire salamanders give birth to fully developed voracious larvae. Adult fire salamanders feed exclusively in the terrestrial habitat and thus the amount and biomass of deposited larvae is not dependant on the carrying capacity of the aquatic habitat. Therefore, the recent habitat adaptation of the fire salamander to pond reproduction brings a new situation to these pond ecosystems, i.e. a pulse of consumers that exert a sudden predation pressure on the food web. This initial high consumer pressure is independent from regulatory mechanisms within the pond and enhances indirect effects that additionally alter the coupling of the aquatic and terrestrial habitat.

Mass mortalities and adaptations to the pond conditions enhance net-flux from forest to pool

In contrast to the situation in streams, where downstream drift of fire salamander larvae can regulate larval densities (Thiesmeier 2004), such a density-regulation mechanism does not exist in the stagnant pond habitats. As a result, high abundances of salamander larvae are maintained for much longer periods of time, and the risk of starvation due to intraspecific competition increases. Such stress conditions probably favour mass mortalities of salamander larvae. However, two sets of critical abiotic conditions were also linked to mass mortalities in pools: The first were strong decomposition processes, as indicated by high ammonia and low oxygen concentrations (below 10% O_2 relative saturation). The low oxygen concentrations are probably the reason for the high mortality, even though other factors associated with decomposition can also contribute to mortality. The second process is an increase in water temperature to 30°C and higher,

something which only occurred in sun-exposed ponds. As fire salamanders were originally adapted to cold, groundwater-fed stream habitats, a tolerance of up to 30°C is already remarkable. Taken together, such pond-specific mortality factors enhance the flux from forest to pool and could result in a 100% net import of salamander biomass into the ponds.

Such mortality factors and other attributes of specific ponds led to a very high between-pond variation with respect to metamorphosis success, ranging from 0 to 22% (W2). At metamorphosis, the pool larvae had increased their body mass by up to seven times that of their mass at birth. Given the average observed metamorphosis success of only 4% in all investigated pond habitats, the direct salamander-mediated export was considerably lower than the import, regardless of hydroperiod and habitat type. Moreover, specific adaptations of the fire salamanders, which increase the chances of survival in the pond habitat, enhance the net-contribution of terrestrial matter to the aquatic system. Weitere et al. (2004) showed that, while larger at birth, the metamorphosis size of the pond-reproducing ecotype of the fire salamander is significantly smaller than the stream breeding ecotype. The consequent reduction of the larval period and energy uptake in the aquatic system is probably an adaptation to reduce the risk of rapid desiccation and to escape the high food limitation of the ponds (Weitere et al. 2004; Johnson and Wallace 2005). Such behaviour would also increase the direct flux of matter from the forest to the pond.

Salamander larvae feeding behaviour indirectly alters subsidy exchange

Aside from the directly salamander-mediated matter flux, our study identified two indirect ways in which salamander larvae alter the flux of matter between the pond and the adjacent forest habitat. One is the consumption of insect larvae, which may have otherwise emerged as adults. While *S. salamandra* larvae in streams feed largely on fully aquatic amphipods (Thiesmeier 1992), insect larvae (particularly chironomids) played an essential role as food items in the ponds (Fig. 1.3), and comprise a significant amount of somatic biomass production. Emerging aquatic insects are a major food source for riparian predators (see above, Baxter et al. 2005; Paetzold et al. 2005), and they play an important role in linking the aquatic with the terrestrial ecosystem. It is shown here, that the biomass of salamander larvae greatly exceeded the biomass of food organisms in several cases. By exerting strong top-down pressure on emergent insects, the insect-

mediated export is reduced, which leads to a decreasing trophic coupling of the aquatic and terrestrial environment. In comparison to longitudinally open stream communities, the closed pond ecosystems are more sensitive towards predator abundances due to their isolated character. Since spatial avoidance and emigration is restricted or simply not possible, and re-colonisation by food organisms (especially insects) takes place only through oviposition events once or a few times a year, the risk of temporal species-specific prey extinction in one habitat is increased (Cooper et al. 1990). Consequently, the insect-mediated flux from pool to forest is significantly reduced by the presence of the salamander larvae.

Another indirect effect is caused by the consumption of terrestrial invertebrates. Our stomach content analyses showed that salamander larvae in ponds could feed on large amounts of terrestrial invertebrates. Such feeding behaviour is well known to a great extent for different fish species (Kawaguchi et al. 2003; Mehner et al. 2005; Kraus et al. 2011) but also for adult and paedomorphic newt species in similar small aquatic ecosystems (e.g. Denoël & Joly 2001; Denoël 2004; for *Mesotriton*). This behaviour is especially beneficial for the amphibian larvae in the small food-limited systems as it provides an additional food source from a terrestrial origin. Unlike terrestrial invertebrates that are trapped in the water and die, the food resource consumed by the salamander larvae is directly introduced into secondary consumer pathways. It is likely that this substitution of food accounts for some of the seemingly paradoxical mismatch of relatively high salamander production at low densities of aquatic food resource.

While the detected predation of terrestrial invertebrates by the salamander larvae represent a pathway with a terrestrial to aquatic flux, the predation of salamander larvae by riparian predators would represent a pathway with a flux in the opposite direction, i.e. aquatic to terrestrial flux. However, predation on larvae by riparian predators should be considered low in our study system, as potential predators such as snakes, water birds and water shrews do not systematically feed on amphibian larvae in this type of ponds. This view is corroborated by our observation that losses of individually tracked larvae at later stages were rare. Altogether, the fluxes towards the aquatic system thus dominate over the fluxes towards the terrestrial system.

Conclusion

Differential aquatic larval habitat choice of terrestrial fire salamanders in Central Europe resulted in a unique situation, in which a voracious top predator is periodically introduced in high abundances to pond ecosystems in spring. Compared to the amount of subsidies through plant detritus (between 200 to 370 g DM m^{-2} yr^{-1}; Lebret et al. 2001; Hansen et al. 2009) the direct residue transfer caused by salamanders is relatively low. However, the effects on food web structure, and consequently the subsidy exchange through other pathways was profound. Accordingly, our data adds three important mechanisms to the function of a top predator for organism-mediated matter flux across habitat boundaries: (1) the indirect effects through predation on emergent insects, (2) the across-habitat predation and (3) the mass mortality due to extreme abiotic conditions in the aquatic system. Future studies should quantify the effects of these mechanisms for other emergent predators to test for the general relevance of these new mechanisms. Our study and the conceptual flux model (Fig. 1.1) should help to identify these mechanisms in specific studies.

2. Chapter

Inter-annual climate variability drives the performance of salamander larvae in ponds through habitat formation and match with prey species

2.1 Introduction

Small ponds are important isolated habitats in the temperate European forest (March and Bass 1995). They function as stepping-stones and refuge for aquatic and semi aquatic organisms (Ebert and Balko 1987). As the formation of these aquatic systems such as ditches, wheel ruts, rainwater puddles depends on the amount of autumnal and spring rainfall and snow melt, their temporal variability with respect to habitat size and hydroperiod is high (Joger 1981; Brooks 2000). Since the ponds inevitably dry out during summer, the time for aquatic organisms to complete their lifecycle in ephemeral ponds is limited and fully aquatic organisms can only survive these insecure conditions by the formation of drought resistant-resting stages or a high dispersal potential. Thus, most inhabitants of those temporal water bodies are semi-aquatic taxa such as insects and amphibians (Rowe and Dunson 1995; Griffiths 1996) that only fulfil their juvenile development in the pond. Nevertheless, the aquatic phase plays a crucial role in the life cycle of those species by significantly altering the success of populations (Semlitsch and Wilbur 1988; Ryan and Semlitsch 2003).

Small aquatic habitats are subjected to a high inter-annual variability of interacting climate factors such as temperature regime and rainfall. These climatic factors in turn control habitat size, food availability, reproductive timing and metabolic activity (Winder and Schindler 2004). The development in pond habitats is constraint by pond drying in summer. Within the restricted time frame, the timing of reproduction and the growth patterns must meet beneficial climate conditions in order to complete the lifecycle successfully (Stenseth and Mysterud 2002). The most influential conditions that determine growth success in poikilotherm species are temperature regime and food resource availability. Both factors often interact since food can only be efficiently exploited when the temperature regime allows for metabolic activity. Consequently, the matching of life history to climate regime is relevant to the efficient exploration of prey

(Edwards and Richardson 2004; Durant et al. 2005; Yang and Rudolf 2010). A mismatch between predators and prey can occur due to different environmental triggers in each species influencing the prey availability for the next trophic level. The influence of match and mismatch is most dominant in species-poor environments and for species in higher trophic positions relying on a single prey species (Durant et al. 2007).

This study investigated the ecological performance of pond reproducing European fire salamanders (*Salamandra salamandra*) over a two year period and especially focused on the influence of inter-annual variation of climate parameters on life history strategies, and ecological performance on both individual and population level. Most populations of the fire salamander in central Europe reproduce in small woodland streams, which have fairly stable conditions (Thiesmeier 2004). Yet, some populations of the fire salamander reproduce partly or exclusively in ponds (Thiesmeier 2004; Manenti et al. 2009), of which many are ephemeral. This pond reproducing ecotype of the fire salamander has been recognised as a recent local adaptation (Weitere et al. 2004; Steinfartz et al. 2007a). In this novel habitat type the larval development faces a complex set of new environmental constrains such as high variation in water temperatures and unpredictable hydroperiod. It was shown that pond ecotype larvae display significantly higher birth weights and a reduced metamorphosis threshold size in order to reduce their aquatic larval phase in the ephemeral environments and escape the habitat before it dries (Weitere et al. 2004; Steinfartz et al. 2007b). However, high developmental rates can only be maintained if the temperature is high enough for metabolic activity and coincides with periods of high prey availability.

The larvae of the fire salamanders are generalist predators and can utilise a wide set of prey organisms. Moreover, the larvae are amongst the top predators in the small fish free habitats, and the food demand of pond larvae is high (chapter 1). As the fire salamander is larviparous (i.e. gives birth fully developed, to relatively large larvae), their initial density is not bottom up controlled by the carrying capacity of the habitat (Thiesmeier 2004; chapter 1). In contrast, the prey organisms in the ponds usually come in single pulses, particularly in early spring. Later in the year, aquatic food resources are quickly overexploited as high abundances of salamander larvae persist (chapter 1). As the food becomes scarce in the ponds the larvae have to acquire alternative food sources. The consumption of food from terrestrial origin could be shown to become important (chapter 1 and 3, Blaustein et al. 2013). Furthermore, under food shortage

cannibalism occurs. Cannibalism is considered a key behaviour in supporting food deprived populations in extreme habitats (Degani et al. 1980; Buckley et al. 2007; Manenti and Ficetola 2011).

It was assumed, that the switch of initially stream reproducing fire salamanders to reproduction in ephemeral ponds needs a large set of flexible adaptations to cope with the highly variable pond environments. Furthermore, the match of essential factors, such as rainfall, temperature regime and food availability should critically control the success of the larval development in this highly dynamic environment. In two consecutive years, the chapter investigated here the effect of the environmental frame (temperature regime, water regime) on the performance of fire salamander larvae in ponds. It was analysed to what degree ecological performance is limited by factors such as temperature regime versus food availability and how individual and population performance depends on a short windows of optimal environmental conditions.

2.2 Material and Methods

During 2011 and 2012 the salamander larvae populations of four ponds (P1-4) that were mostly dependent on autumnal/winter rainfall (autumnal pools) were investigated. The ponds were ephemeral, had a surface area of between 0.5 and 14.1 m^2 and a depth between 1 and 1.5 m with high amounts of leaf litter and other plant detritus covering the pond bottom (Table 2.1). The sites P1 and P2 could be considered consistently throughout the two years. The extremely dry winter 2011/2012 prevented the third pond (P3) from refilling and no salamander larval deposition could be observed in 2012 here. Thus it was also considered the site P4 to the analysis in 2012, as this site was similar to P3 with respect to size, depth and macroinvertebrate composition. The ponds P1 and P2 were also used in an experimental setup on the impact of salamander larval presence in ephemeral ponds and separated into enclosure and exclosure areas (see Chapter 3). Only the enclosure segments where considered here Care was taken to avoid altering initial larval densities in these segments in comparison to unmanipulated ponds.

Study sites

All studied breeding ponds are located in the Kottenforst close to the city of Bonn, Germany (50°40'4''N, 7°1'22''O) (Steinfartz et al. 2007b). This 40 km^2 large, old broadleaf mixed forest is relatively isolated and lies on a topographic plateau between the cities of Bonn and Meckenheim, with a highway dividing the area into two main parts. Oak (*Quercus spp*.), European beech (*Fagus sylvestris*) and spruce (*Picea spp.*) are the dominant tree species of the area. The soil is stagnosolic, therefore periodically allowing the formation of various puddles and marsh areas in ditches and depressions. A high proportion of the small aquatic habitats in the area are of anthropogenic origin (Second World War bomb craters, roadside ditches and machine tracks). In this environment about 50% of the salamander population used these small stagnant waters as reproduction habitats while the rest used small first order streams on the sides of the plateau. The latter is considered the typical habitat for salamander larvae (Thiesmeier 2004).

Temperature, thermal time and hydroperiod

In order to calculate the amount of water, the surface area was measured every 4 weeks; water depth could be observed via a levelling staff in the pond centre. Temperature regimes were monitored using temperature data loggers (Ebro EBI 20 at 2 h measuring interval) in a water depth of 10 cm in the centre of the pools. The measured data points were added into a day unit for consistency. In order to compare the temperature regimes and resulting growth potential in the two years we calculated thermal time (TT, °C d), i.e. the sum of physiologically effective temperature (T_e, °C) over a time interval (t_1 until t_n) during developmental time (t, d) (see Trudgill et al. 2005).

$$TT = \sum_{t_i = t_1}^{t_i = t_n} (T_e \times t_i) \qquad (7)$$

The physiologically effective temperature (T_e,) is herby calculated by the difference between the minimal temperature necessary for growth (T_{min}) and the actual temperature (T_0). T_{min} temperature, as well as the growth kinetics in response to temperature, was calculated from earlier laboratory experiments (M. Weitere, unpublished data, see appendix). Thermal time was calculated from the daily mean temperature during the entire larval period.

Further it was estimated the theoretical amount of thermal time to complete metamorphosis. Mean weight at birth is 0.17 g fresh mass and the mean weight at metamorphosis was 0.77 g after metamorphosis (Weitere et al. 2004). Weight loss at metamorphosis is approximately 10% (Gasche 1939). Thus it was assumed that the larvae had a fresh weight of 0.84 g right before metamorphosis. The amount of thermal time necessary to complete metamorphosis under unlimited food conditions was calculated as reference for the growth and development time in the ponds (see appendix).

Larval deposition and growth

Unlike all other central European amphibians, the fire salamander is larviparous, thus give birth to fully developed larvae in a size range of 27 to 35 mm (Thiesmeier 2004). Larval deposition usually occurs discontinuously during rainy nights in early spring (Thiesmeier 2004; Weitere et al. 2004). All larval deposition events in the years 2011 and 2012 were monitored by counting approaching females at night when temperature and rainfall was sufficient. The larval densities were estimated the next day by the capture-recapture method (Youngs and Robson 1978). For this, as many larvae as possible were caught within ten minutes using a standardised triangular amphibian scoop net with 50 cm width at the base and a mesh size of 0.5 cm (Schlüpmann et al. 1995). All larvae were sorted into age cohorts and marked by digit amputation according to their age cohort, with each age cohort receiving a distinct toe clip pattern. Afterwards they were released back into the pond immediately, and recapturing was conducted 24 h later. The total number of individuals (N) in each pond was calculated by the number of marked larvae (m) times the number of recaptured larvae (c) divided by the number of marked larvae among the recaptured larvae (r) by the following equation (Youngs and Robson 1978).

$$N = \frac{(m x c)}{r} \tag{8}$$

Marking and recapturing of larvae was conducted after every deposition event. In-between and after the reproduction peaks, the number of larvae caught within ten minutes ("catch per unit effort") was used to monitor the population development and assess the survival of the different cohorts and weigh the larvae to estimate growth. (for further details see chapter 1). During the breeding season, recapturing was conducted every month (in 2011 on the 22/03, 03/04, 28/04, 22/05, 05/06, 25/06 and 06/07 and

in 2012 on the 06/04, 25/04, 08/06, 28/06 and 07/07). Weighing and recapturing also yielded monthly biomass estimates. Salamander fresh mass was converted to dry mass using fresh to dry mass correlations after Crump (1979).

Estimate of food availability

Both the macroinvertebrate and zooplankton communities were sampled monthly in each pond, in order to estimate the standing crop of food organisms available to the salamander larvae. The organisms were classified as potential salamander food, i.e. either as edible or inedible according to previous investigations of salamander larvae gut contents (Weitere et al. 2004). Only the edible proportion of the pond fauna was considered. For a detailed description of criteria for food organisms see Weitere et al. (2004). The body size of all sampled individuals was measured and the dry mass (DM) was estimated for all organisms using size-body mass correlations (Dumont et al. 1975; Rogers et al. 1976; Rogers et al. 1977b; Benke et al. 1999).

Cannibalism

Systematic cannibalism in fire salamander larvae is usually a result of extremely food-limited conditions (Reques and Tejedo 1996). Cannibalistic larvae show larger body size and are generally well fed even though the majority of the larval populations display poor body conditions (Degani et al. 1980). In order to monitor cannibalistic behaviour in the larval population we systematically screened all captured larvae for potential cannibals by 1) direct observation of cannibalistic behaviour, 2) screening for large larvae and provoke regurgitation (commonly large food items were already regurgitated in the dip net after catching). All regurgitated prey was measured and cannibalistic individuals were marked specifically with toe clipping for further monitoring. Additionally, large individuals were sampled for gut content analysis specifically.

2.3 Results

Hydroperiod, habitat size and temperature regime

All investigated pools are autumnal pools that are refilled mainly during autumn and winter rainfalls or snowmelt before the vegetation period starts. Autumn 2010 allowed the ponds to be filled with water for the reproduction season of 2011. The ponds had an area of between 12.6 m^2 (P1) and 14.1 m^2 (P2) and the hydroperiod of the ponds P1 and

P2 lasted till August while P3 dried completely in June. In the autumn/winter of 2011 the ponds were only slightly refilled, thus the same pools had small surface areas in 2012 (4.6 m^2 for P1, 5.0 m^2 for P2, Table 2.1). The pool P3 did not refill with water at all, thus I included a fourth pool (P4) with a surface area of 5.0 m^2 into the investigation (Table 2.1). In 2012, the hydroperiod of P4 was until July. As the habitat was smaller and, at the same time, more larvae were deposited in 2012, the initial salamander densities were distinctly higher with 52 (P4) to 143 (P1) ind. m^{-2} in 2012 compared to 3.4 (P3) to 37.9 (P1) ind. m^{-2} in 2011. The larvae number ranged between 260 (P4) and 701 ind. $pond^{-1}$ (P1) in 2012 compared to 47 (P3) to 478 (P1) ind. $pond^{-1}$ in 2011 (Table 2.1).

While 2011 started with lower temperatures (compared to 2012) during the time of first larvipositioning, the temperatures steadily increased to a mean temperature of 9.3 °C in mid March. 2012 had a warmer start, but a distinct drop in temperature during the first developmental months of March and April, with mean temperatures of 7.7 °C. The accumulation of thermal time was also lower during March/April 2012 compared to 2011 (Fig. 2.1).

We found that fire salamander larvae need 326 °C d to reach the mean metamorphosis size of 0.84 g fresh mass (see supplementary data). This sum of thermal time was reached in both years by the end of May (Fig. 2.1). Approximately at this time the first metamorphs could also be observed (Table 2.1).

Table 2.1: Comparison of pond size, salamander larvae phenology and density in the four ponds and the two years. The salamander population was not estimated (n.e.) in P4 in 2011. In 2012 no larvae were deposited in P3.

Pond Area (m^{-2})			Date of first Larvipositioning		Initial larval abundance (ind. $pond^{-1}$)		Metamorphosis success (ind. $pond^{-1}$)		Date of first Metamorphosis	
	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012
P1	12.6	4.9	25/03	12/03.	478	701	11	9	02/06	22/05
P2	14.1	5.0	25/03	12/03	173	331	7	9	20/06	22/05
P3	13.8	0.5	25/03	-	47	0	0	0	-	-
P4	15.0	5.0	n.e.	12/03	153	264	n.e.	0	n.e.	-

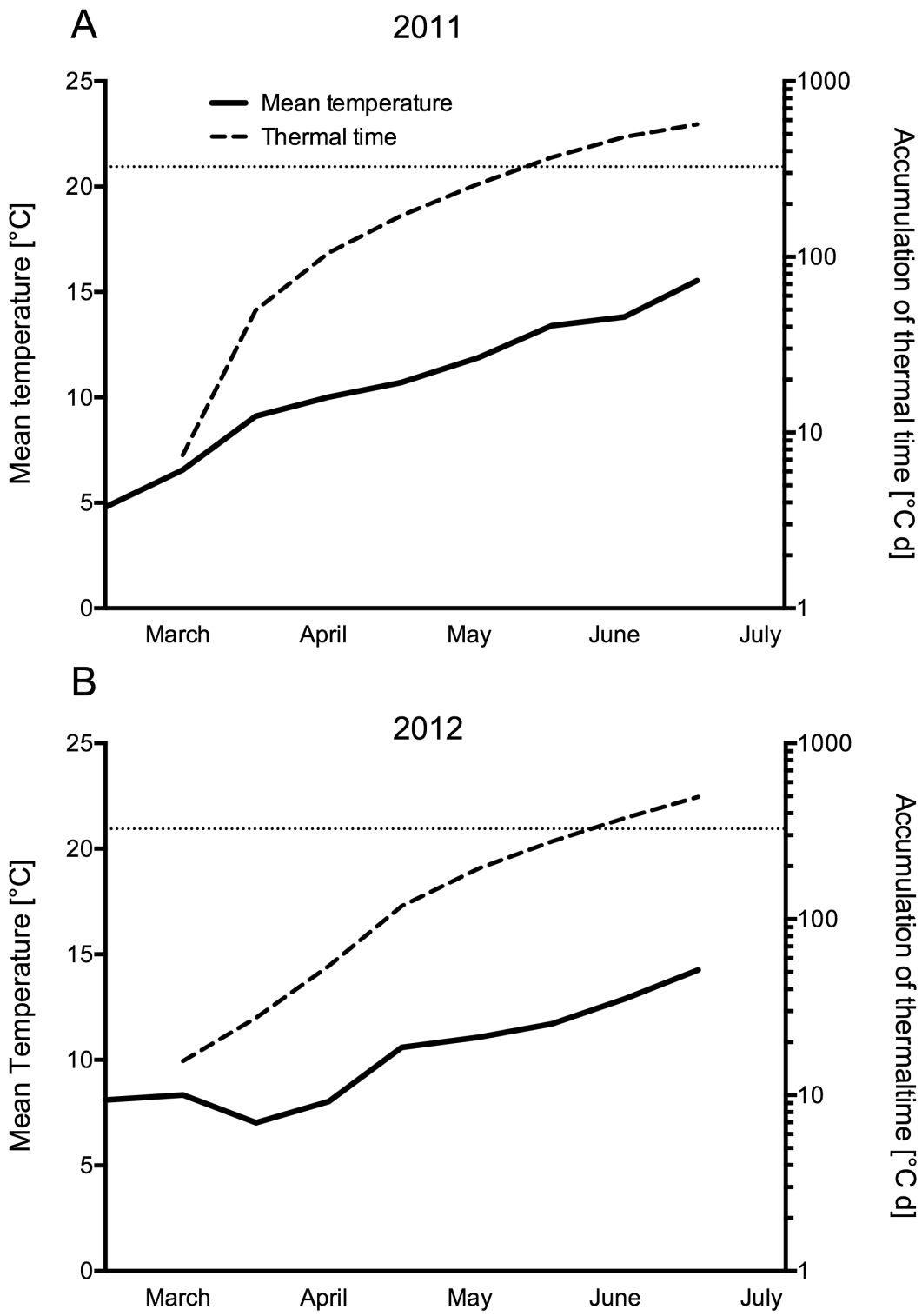

Fig. 2.1: Daily mean temperature trend (solid line) of three pools and biweekly accumulation of thermal time (dotted line) in 2011 (A) and 2012 (B). The horizontal dotted lines represent the theoretical minimal thermal time requirement of newly hatched salamander larvae with a mean body mass of 0.17 g to reach mean metamorphosis size of 0.84 g (see results).

Food availability

Culicid larvae, predominantly larvae of *Aedes vexans*, comprised up to 99% of all macro-invertebrate biomass in early spring from March to May. The amount of food biomass exceeded the biomass of salamander larvae in all ponds in March and April and up to 50 times in P1. In 2011 between 0.9 and 6.6 g DM m^{-2} culicid biomass was available for consumption by salamander larvae (Fig. 2.2). By mid May the culicid larvae were metamorphosing and left all ponds. With that, the total food biomass decreased strongly and the biomass of salamander predators greatly outweighed the total biomass of the prey organisms. Plankton crustaceans, beetle larvae and chironomids dominated the pond macro-invertebrate fauna in summer (Fig. 2.2).

Few culicid larvae hatched in 2012, so the major peak of food organisms did not occur in this year, yet the biomass of present salamander larvae was even higher than in the previous year (see above). Thus, the salamander biomass exceeded the available food biomass during the entire year. Maximum food biomass occurred in P1 in April with only 0.25 g. The summer food biomass and composition did not differ significantly from the previous year (Mann-Whitney U-Test $p>0.05$). The salamander abundance remained distinctly higher in 2012 when compared to 2011.

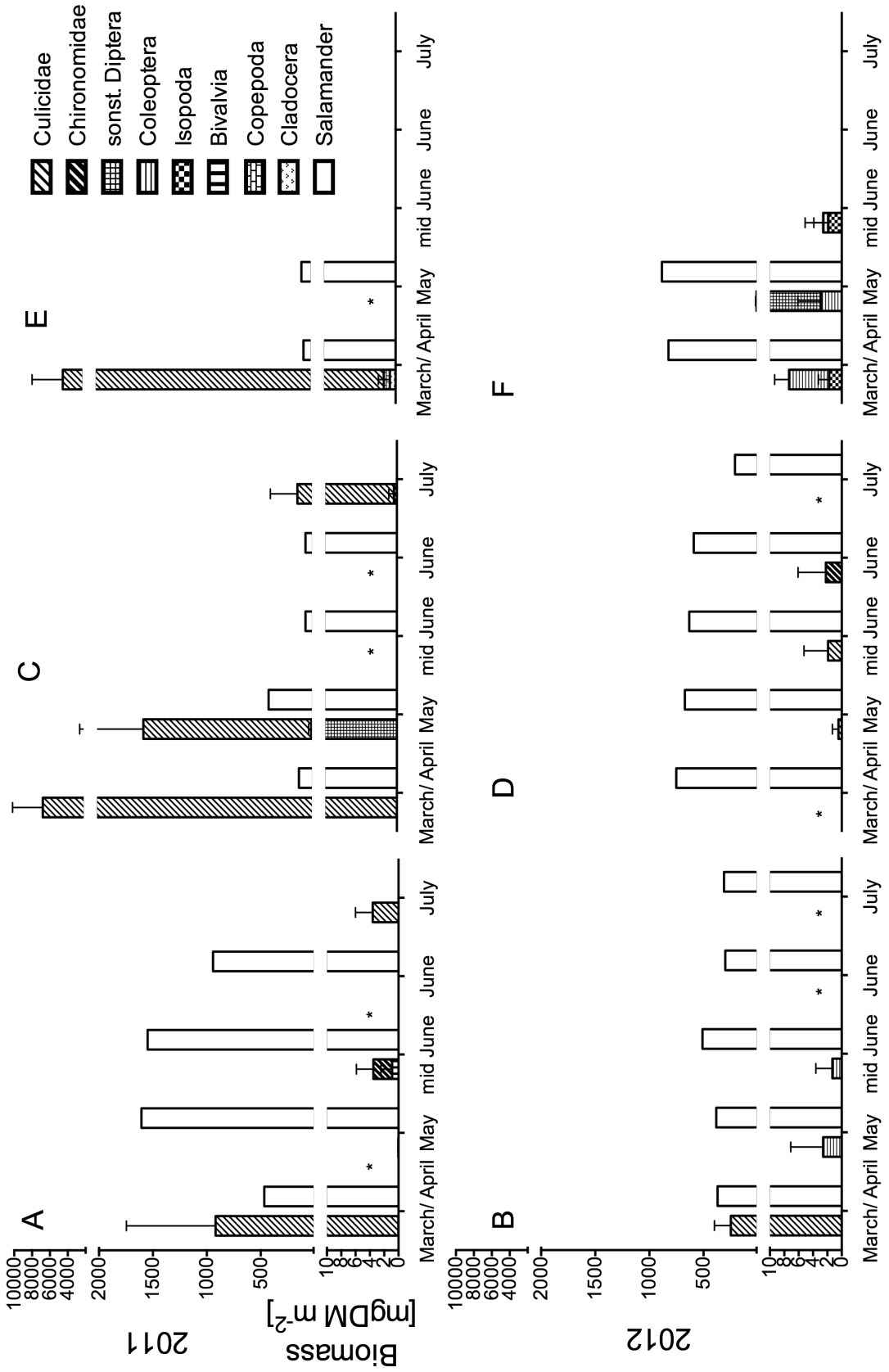

Fig. 2.2: Standing crop dry mass of food organisms versus dry mass of salamander larvae in ponds P1-P4 in the two years (mean of three samplings per sampling occasion ± SE). Samples indicated with * contained less than 0.0005 g of food crop biomass. P3 and P4 dried in mid June and June, respectively (no samples at those times).

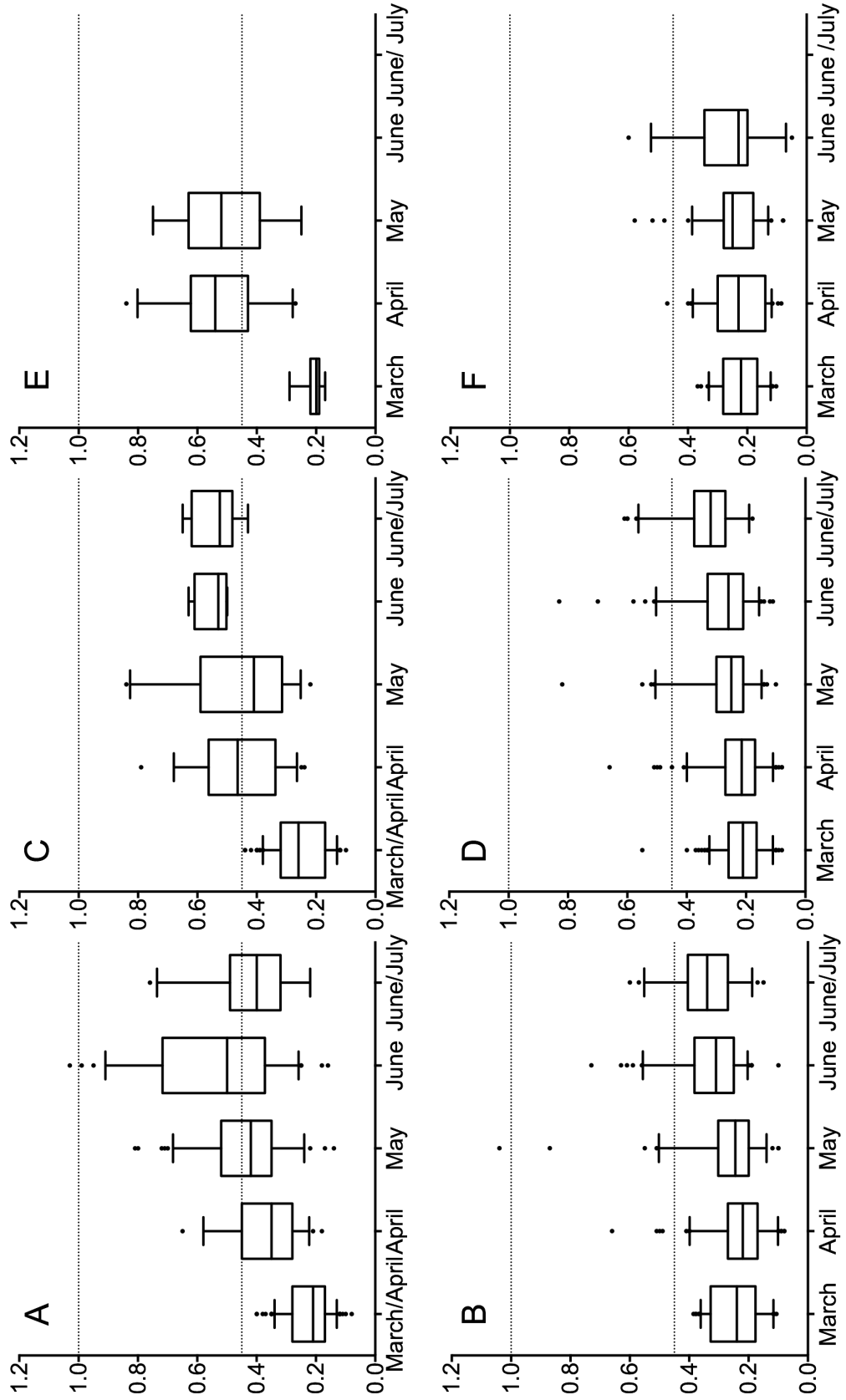

Fig. 2.3: Size distribution of salamander larvae in different ponds (A, B) P1 (C,D) P2, (E), P3, F (P4) throughout two investigated years. (A, E, C) 2011, (B, D, F) 2012. Boxes represent median and quartiles; whiskers represent 5 and 95% percentile. Individual sizes above the 95% percentile are indicated by aligned dots. Dotted lines represent max. - min. size range for metamorphosis of 0.44 to 1.0 g fresh mass (see results).

Growth of salamander larvae

The main biomass gain of the salamander larvae took place in March and April. Starting at a mean weight at birth of 0.2 g, the mean weight of the larvae quickly doubled within this first month of development in 2011 (Fig. 2.3). The time of highest growth increment coincided with the time of highest prey biomass in 2011. The mean growth rates in the ponds during the month March/April 2011 ranged between 0.019 d^{-1} ± 0.007 SD (P3) and 0.028 d^{-1} ± 0.003 SD (P2) (Fig. 2.4). In contrast to 2011, the growth in the same timeframe in 2012 was low. Growth rates ranged between 0.007 d^{-1} ± 0.004 SD (P1) and 0.011 d^{-1} ± 0.007 SD (P2) for the first growth period in March/April in 2012 (Fig. 2.4). This low growth in the early phase of 2012 could be attributed to lower temperatures rather than lower food concentrations: In all ponds in both years, the mean growth rate in the early season was equal (or even higher) than the theoretical maximum growth rate for the mean temperature calculated from laboratory experiments under constant temperatures and unlimited food availability (Fig. 2.4). Later in the season the mean growth increment decreased strongly in all ponds.

In 2011, the body mass of the salamander larvae showed less variability than in 2012. During all sampling dates in 2011 the weight difference between the 95% quantile and the heaviest individuals was low. The heaviest individual was approximately twice as heavy as the median (1.0 g in relation to a median of 0.6 g in P1 in June; Fig. 2.3). In 2012 the growth increment did not change significantly with time and the population mean weight did not vary distinctly from the birth weight until the ponds dried out in July. Nevertheless, from late April onwards, single individuals of the population had increased their body weight to more than the minimal observed weight at metamorphosis of 0.44 g. This group of fast growing individuals could be up to 4 times heavier than the median (e.g. 1.0 g opposed to a population median of 0.3 g in P1 in May) (Fig. 2.3).

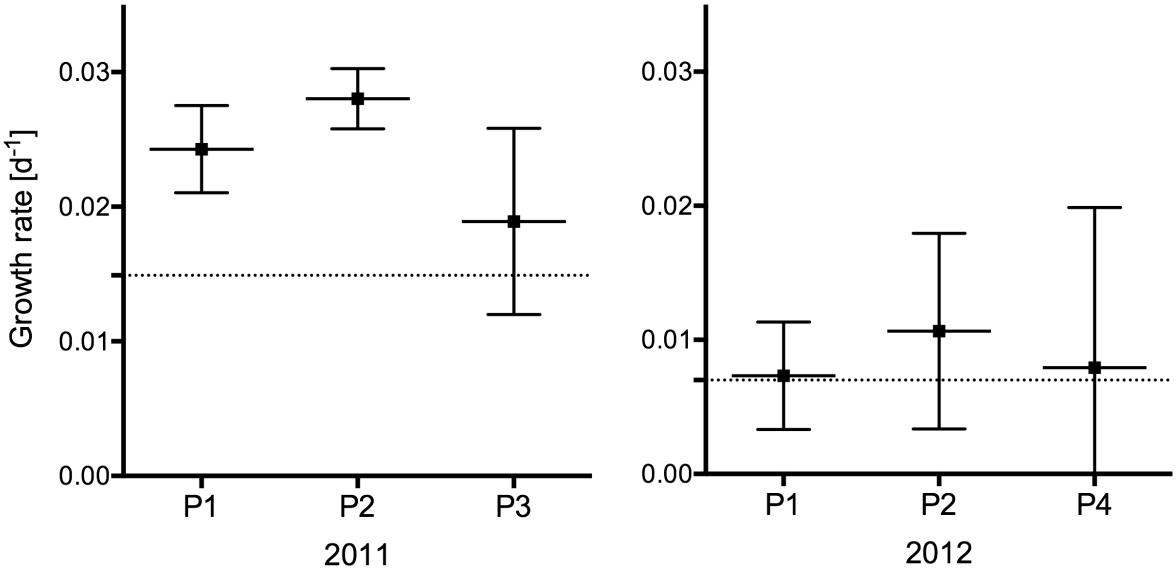

Fig. 2.4: Mean growth rate of salamander larvae during the early larvae phase in April (mean and SE). Dotted lines indicate the estimated growth rate at mean temperature for April (9.3°C in 2011 and 8.1°C for 2012 respectively) and ad-libidum food supply based on laboratory growth measurements.

Cannibalism and cohort survival

Within the large individuals of 2012, several cases of cannibalism could be observed. A total of 10 independent cannibalistic incidents (1 in P4, 3 in P2, 6 in P1) were recorded. They were confirmed by regurgitated of salamander prey (7 cases), gut content screening (2 cases) and direct observation (one case). Cannibalism was only observed in individuals heavier than 0.6 g and those individuals managed to consume conspecifics up to half of their own body length. The prey salamander frequently belonged to the smallest cohorts. The large range in size could only be observed in 2012 and not in 2011. No cannibalism was observed in 2011 despite similar observations effort compared to 2012.

In early spring, every event of strong night rain at temperatures over 15°C resulted in a larvipositioning event. The larger the intervals between rain events, the stronger the size difference between the larval cohorts was. The larvae could be assigned to a certain larvipositoning period during early March and late April. At least 100 individuals of every cohort were marked in order to follow cohort growth and survival. In both years 80% of all depositing females arrived at the ponds in March to dispose their larvae. While in 2011 all larvae were born in several deposition events within a short time

period between March 25th and April 4th, the deposition events were more distinctly separated by periods of dry weather in 2012. Deposition took place in the nights of the 12th and 20th of March (Table 2.1). Additionally, one month later on the 14th of April, the larval density was further increased by another deposition event (Fig. 2.5). All fast growing large individuals in 2012 belonged to the first two cohorts of the year. In contrast, no individual of last age cohort could be recaptured four weeks after birth; the entire cohort had disappeared, while the contributions of the other age cohorts remained roughly constant (Fig. 2.5).

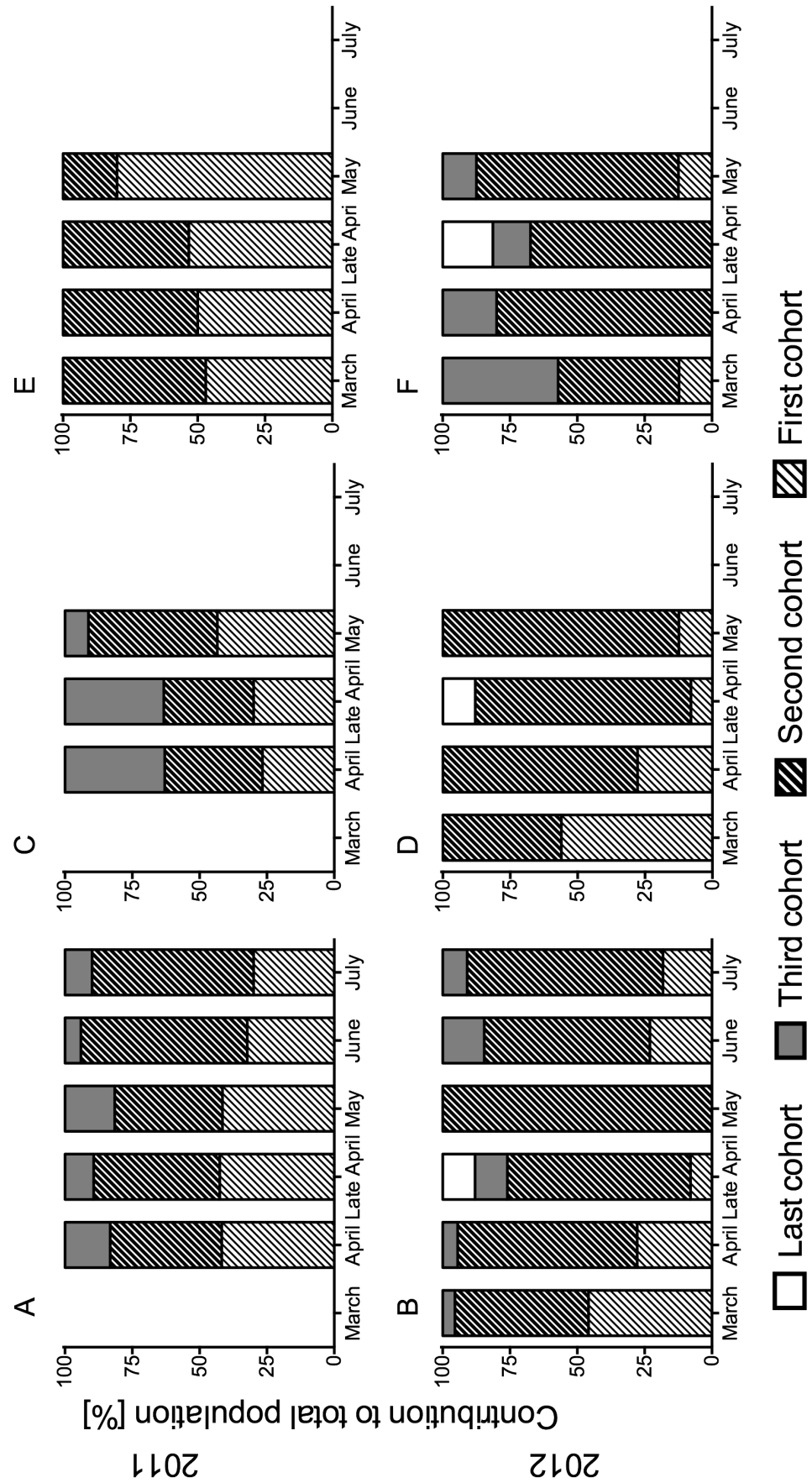

Fig. 2.5: Cohort composition based on marked recaptured individuals in different ponds (A, B) P1(C, D), P2, (E) P3, F (P4) throughout two investigated years. (A,E, C) 2011, (B, D, F) 2012 The first, second and third cohort were born between 01/03 and 30/03 in both years; the last cohort was born after15/04 in 2012. This last cohort disappears within two sampling occasions after it was born.

Metamorphosis success

At metamorphosis the larval weighted between 0.4 g and 1.0 g fresh mass. The first individuals reached this weight already by the end of April in both years (Fig. 2.3). Nevertheless metamorphosis could not be observed before the end of May in 2012 and early June in 2011 (Table 2.1). Metamorphosis period lasted until pond drying between June and August. Neither the amount of larvae that left the water in three ponds (14 larvae in 2011 and 16 larvae in 2012), nor the mean weight of fresh metamorphosed juveniles (mean of 0.58 ± 0.12 g fresh weight in 2011 versus 0.59 ± 0.24 g fresh weight in 2012) differed significantly between the years (Mann-Whitney U-Test $p>0.05$; Table 2.1). In 2012, metamorphosing individuals were larvae born during the first and second larvipositioning event. In contrast, in 2011 larvae from equally late and early birth dates were able to metamorphose.

2.4 Discussion

Direct and indirect effects of the climate framework on the performance of the salamander larvae

Ephemeral pools are habitats with high annual variability in abiotic conditions. The pattern of abiotic conditions and their occurrence strongly dictates species composition and population dynamics in ephemeral ponds. Species need to fulfil their development in the pond within a restricted time frame, which requires optimal match of demands to environmental settings. The match-mismatch hypothesis (Cushing 1969) in the strict sense refers to trophic relationships. Generally, a match or mismatch between predators and prey species abundance can occur if different environmental triggers (e.g. photoperiod and temperature) regulate the phenology of the two species. Predator and prey populations will match when the two triggers lead to co-occurrence of both whereas a mismatch can occur when the environmental setting results in a different triggering of life cycles in predator and prey. The latter case results in a lack of resources for the consumers (Durant et al. 2005; Durant et al. 2007).

Here it was shown that variation in the interplay of climate factors, such as hydro-period and temperature, can strongly alter the interaction of the main prey and predatory salamander larvae: While the amount of food items for salamander larvae in the ephemeral ponds is generally low and restricts larval growth (Weitere et al. 2004), one

abundant prey species (the mosquito *Aedes vexans*) can develop in the forest ponds in high densities in early spring. Hatching of this mosquito species is most strongly correlated to the hydrology of the forest ponds, and undergoes strong population fluctuations which are dependent on the water refill. *Aedes* species deposit eggs in the wet soil above the pond's waterline, and the larvae only hatch if the water level rises above this horizon in the next year (Peus 1972). In high rainfall years the culicid larvae hatch in a high abundance and provide the most abundant food resource for salamander larvae in the early season as recorded here in 2011. In the low rainfall year the lack of refill in the preceding year prevents the build up of a large culicid population (as recorded here in 2012) and reduced the crucial initial food resources for the young salamander larvae. Thus the climatic frame can affect the food density.

However, even though culicid abundances differed distinctly between the years, it demonstrated another important factor, i.e. the temperature conditions during prey peak. The data show that salamander growth was temperature, rather than food, limited during the early spring phase in both years. In both years, the larvae showed maximal growth rates for the particular temperatures. They reached, or even exceeded, growth rates as estimated in the laboratory experiments under unlimited food conditions for the particular temperature. The higher growth rates are probably a result of patched temperature conditions in the pond and the larval behaviour. They are often found in the warmer surface layer, whereas the temperatures were measured at a depth of 10 cm. Nevertheless, the high growth rates do not indicated food limitation during the early spring. However, in 2012 temperatures were distinctly lower than in 2011, and the salamander larvae were not able to efficiently use the prey peak. In 2011, 105 degree-days of thermal time were available during the peak of food availability until the culicid larvae emerged from the ponds in early to mid May. In 2012 salamander and culicid larvae coexisted for only 54 degree days until the culicids emerged from the ponds by the end of April, due to the low mosquito abundance some time prior to 2011. Our estimate shows that, on average, 326 °C d are necessary to complete metamorphosis if the food supply is not a limiting factor. In 2011, the early larvae could develop to metamorphosis size in time for peaks of high food availability. This early growth conditions is beneficial, as the larvae suffer from severe food limitation as soon as the culicid larvae emerged in May, and thus cannot benefit from warmer summer temperatures (see also Weitere et al. 2004 for negative effects of high temperatures

under food limitation). It is consequently not sufficient that the occurrence of the predator is matched to the abundance peak of the prey; growth conditions during prey peak with respect to temperature are also important.

Flexibility in ecological performance in response to inter annual climate variation

Considering the limited food conditions after the metamorphosis of the culicids, which strongly limit larval growth (see also Weitere et al. 2004), high growth during the early phase appears essential for metamorphosis success. Nevertheless, a reduced metamorphosis success in 2012 compared to 2011 could not be shown. Apparently, the mismatch in the early larval phase could be compensated with different life history strategies. In the year with a good matching of salamander and culicid larvae (2011) the majority of the population increased in size until the food resources were exploited. In the year with low matching of food and predator (2012) bimodal size structuring of the population could be observed, i.e. one large group of small individuals that comprised the majority of the population, and rare single large individuals that constantly increased in body mass. This split greatly increased throughout the month and only the large individuals metamorphosed. Bimodal size structures in populations are often observed when food resources are limited and usually correlated with intraspecific regulation effects such as exploitative competition and inter cohort predation (Claessen et al. 2000; Cohen et al. 2006). The present study could demonstrate repeated cases of inter cohort predation by larger on smaller individuals in 2012. Inter-cohort predation occurs frequently in situations of food scarcity in a variety of species (Claessen et al. 2000; Byström 2006) and is then often crucial to ensure any recruitment of a population to a later life phase (Huss et al. 2010). In fire salamanders cannibalism has been reported in various systems such as caves and ephemeral pools (Degani et al. 1980; Eitam et al. 2005; Manenti et al. 2009). It is usually triggered by low food supply and high population density (Degani et al. 1980; Reques and Tejedo 1996). Consuming conspecifics can fulfil two functions: Firstly, a high value and abundant food resource can be used and secondly, the number of competitors of the same species and consequently intra-specific competition are reduced (Huss et al. 2010). Here, cannibalism was shown to be one flexible strategy to maintain metamorphosis success when prey could not be sufficiently utilised.

Our data also suggest that besides food scarcity and larval density, the cohort distribution plays a significant role in supporting cannibalism. The distribution into age cohorts is dependent on the temporal pattern of rain events during the reproduction season. For the pond reproducing ecotype, optimal rainy nights for deposition in early spring occur sporadically and the females arrive at the breeding sites rather synchronously. The basis for a size split in the population in 2012 is likely founded by the split in birth date between early and late cohorts, which lead to distinct size differences in the larvae (see also: Cohen et al. 2006). In this year, the ingestion of conspecifics was observed only amongst the large individuals. Pressure through inter-cohort predation was particularly large for the latest cohort (those had the biggest size difference to the largest cohort). No marked individual from the last cohort reached metamorphosis. Large size differences as found in 2012 thus probably promote cannibalism and select for large (early) individuals.

Implications for evolution of larviposition timing in ponds

The pond-reproducing salamanders arrive at the breeding sites in some years as early as the beginning of February (personal observation) with a peak of reproduction in mid and late March. Similar early reproduction periods have also been noted for other pond reproducing fire salamander populations in the same climate (Weitere et al. 2004). Larvipositioning was completed by early April whereas females of stream reproducing fire salamanders have their peak of reproduction usually by mid/ end April to mid May (Thiesmeier 2004; Steinfartz et al. 2007b). Thus the stream ecotype reproduce more than a month later than the pond ecotype and their larvipositioning occurs over a longer period of time (sometimes as late as mid June). The typical stream breeding fire salamanders are generally adapted to a stable set of environmental conditions. In early spring flooding events after snow melt and flush floods as a result of heavy spring rains are common and cause severe mortality in larval salamander populations of the small streams and thus will set the earliest timing for a successful reproduction. The hydroperiod of the small headwater streams is continuous and food organisms are abundant at any times of the year. Consequently the selective pressure on stream reproducing salamander females can point towards a later reproduction. In contrast, we identified two factors that should result in large selective pressure towards early reproduction in the pond ecotype of the same species. Firstly the need to match early

peaks of high food availability provided the temperature conditions are favourable. Secondly the strong priority effects by cannibalism on late cohorts if the larvipositioning was discontinuous. Females with a later deposition phase would have a low (if any) reproductive success regardless of larval birth size either because their larvae will not profit from the early abundance of prolific food organisms or because of inter-cohort predation.

As a result, pond ecotype females also seem to be more selective when choosing a suitable place for their offspring and avoid ponds with conspecific larvae (Eitam et al. 2005). While stream females in the laboratory dispose their larvae on a single night, pond females could be shown to split their offspring into different deposition events (Caspers et al. in prep). This bet hatching strategy could be an adaptation towards avoiding priority effects by seeking out uninhabited ponds if possible, especially if the larvae could not be born early enough (Caspers et al. in prep).

Table 2.2: Comparison of environmental triggers, resulting habitat settings and phenological responses of salamander larvae between the two years.

	Year 2011	Year 2012
Prior autumn/winter rainfall	high	low
Habitat size	large	small
Salamander larval densities	low	high
Culicid abundance	high	low
Distribution of spring rain events	even	disrupted
Salamander larvipositioning	continuous	disrupted
Size difference between 1st and last cohort	small	large
Temperature during early development	high	low
Match with early Culicid food pulse	efficient	inefficient
Early growth	high	low
Cannibalism	not detectable	common
Size distribution of Salamander larvae	continuous	bimodal

Conclusion

Variations in climate between different years require flexibility in life cycles for all species in ephemeral pools. For pond reproducing salamander larvae low amounts of food in the ponds were shown to be the main limiting factor. Here it was demonstrated how the species that is originally adapted to stable environmental conditions in stream ecosystems can adapt to a difficult environmental settings and compensate for poor matching with food supply. The matching between predator and prey was demonstrated

to be not only determined by abundance overlap, but also by the temperature conditions allowing for efficient resource usage. (Table 2.2) Mismatch phenomena are increasingly debated in connection to ongoing climate changes (Edwards and Richardson 2004; Hoffmann and Sgrò 2011; Ovaskainen et al. 2013) and the identification of relevant environmental settings as well as species life cycle strategies to adapt to the novel conditions are of importance in this context. Furthermore we could demonstrate how two factors, i.e. food availability and priority effects, select towards early reproduction in pond ecotypes, and should have an effect on the micro-evolutionary split separating the pond ecotype from the main stream breeding population (Steinfartz et al. 2007b).

3. Chapter
Limited effects of top-predators in a highly subsidised aquatic food web

3.1 Introduction

Consumer-resource interactions are important drivers of community dynamics and biodiversity in natural communities. Top predators can control prey biodiversity, abundance and productivity, food web structure and subsidy exchange (Fretwell 1987; Schmitz and Suttle 2001). Some of these top-down effects are the result of direct consumption, e.g. reducing prey abundance, changing population size structure or individual behaviour. Others are effects that cascade down to lower trophic levels and radiate through the food web. Mechanisms like meso-predator release (Brodin et al. 2007; Ritchie and Johnson 2009) or the reduction of competition and thus increasing resource availability as well as nutrient recycling can hereby increase the productivity of different trophic levels (Vance-Chalcraft et al. 2007).

Biotic control in aquatic ecosystems has so far been studied in extensively and the findings are applied in biomanipulation of lakes and freshwater reservoirs (Fretwell 1987). In permanent freshwater ecosystem, predatory fish usually play this role of top predators (e.g. Katano et al. 2013) whereas in small ephemeral water bodies fish are usually absent. In these systems urodelans and their larvae are important consumers and influential top predators (Holomuzki 1989; Holomuzki et al. 1994; Blaustein et al. 1996). Still, often the most influential factor determining abundance and community composition in ephemeral ponds is the limited persistence of the aquatic habitat. For species in ephemeral ponds the risk of pond drying is omnipresent and together with events of oxygen depletion and accumulation of harmful residues the environment is highly stressful (Williams 1996). The environmental stress of the harsh conditions may in turn limit the population size and growth of all populations and reduce the intensity of biotic interactions (Chesson and Huntly 1997, Peckarsky et al. 2008). Under stressful conditions the restrictive framework of abiotic parameters could in fact be shown to be responsible for weaker consumptive effects through top predators (Greig et al. 2013). Anyhow, experimental quantifications of the intensity effects of abundant top-predator species are still rare.

Previously we demonstrated that the larvae of pond breeding fire salamander are the dominant predator in terms of abundance and biomass in some ephemeral pond ecosystems (chapter 1). In several cases the predator biomass will surmount the available food biomass by several orders of magnitudes resulting in an inversion of the trophic pyramid. Further, gut content analyses revealed that fire salamanders consume significant amounts of insect larvae but were also subsidised by terrestrial prey. Based on stomach content analyses, it was calculated that the food demand of the salamander larvae population in ponds should be substantially higher than the macro-invertebrate resource availability. This conjecture implies that the prey population should be substantially top down controlled in ponds where salamanders are present. Here we conducted an ecosystem manipulation experiment to test the outcome of the calculations experimentally. We excluded salamander larvae from one half the pond in two ponds and two successive years to test the hypotheses that (1) salamander exerts a strong top-down control on macroinvertebrate communities, (2) the top down effects are species - specific, depending on their phenology, (3) subsidisation of the food web by terrestrial resources stabilises the salamander population development despite limited supply of pond-dwelling food organisms. Finally (4) trophic diversity and omnivory is assumed to increase when a top predator is absent. Consequently the food web topology is altered in the presence of a dominant top predator.

3.2 Material and methods

Study site

The studied pond breeding population of fire salamanders is found in Kottenforst close to the city of Bonn, Germany. This pond reproducing ecotype could be shown to be a very recent local adaptation (Weitere et al. 2004; Steinfartz et al. 2007b). In the investigation area about 50% of the salamander population use these small standing waters as reproduction habitats while the reminder use small first order streams on the sides of the plateau. For details on the study area and locations see chapter 2.

Exclosure experiments

In the beginning of 2011, two ponds were manipulated to create exclosure and enclosure areas. We used two bomb crater ponds (P1 and P2) that had a nearly circular shape and divided these into two equal parts (each 12.6 (P1) and 14.1m^2 (P2)) with

impermeable PVC foil. The foil barrier was buried approximately 5 cm deep into the pond substratum and emerged the same height from the water surface to separate the invertebrate fauna of the now separate water bodies. Separation was established prior to larvipositioning in February 2011 and after every depositioning all salamander larvae were removed from the exclosure within the next day. The removed larvae were distributed to other, not considered, water bodies to keep natural densities in the enclosure compartments. After the females finished repositioning (despite rain events no observed fresh larvae) the ponds were enclosed with a 60cm high amphibian fences by April to prevent the immigration of adult newts and catch the metamorphs in summer. The experimental runs were started in March.

In 2012 the same ponds were used for a second experimental run. Due to a lower winter rainfall the resulting ponds had smaller surface area in 2012 (4.6 (P1) and 5m^2 (P2) per segment). In the preceding winter the foil barrier was rotated by 90°. The ponds dried out in summer 2011 and froze over in the following winter. Additionally, the barrier was reset for the second experimental run, thus we consider the 2011 experiment as separate replicates.

Larval deposition and growth

In order to asses the initial salamander larval densities in the enclosure compartments, we used the recapture method (Youngs and Robson 1978). For details see previous chapter (Chapter 1 and 2). All captured larvae were weighed, measured and marked by digit amputation. Afterwards they were released back into the pond immediately and recapturing was conducted 24 h later. To screen for population fluctuations and estimate the later population size ,we used time per unit afford sampling and sampled larvae for ten minutes using a standardised triangular amphibian scoop (Schlüpmann et al. 1995). All captured larvae were weighed and measured again to calculate size development. Salamander fresh mass was converted to dry mass using fresh to dry mass correlations after Crump 1979.

Macroinvertebrate density and composition

Benthic macroinvertebrates and zooplankton from all ponds were sampled monthly with three replicates per sampling in order to estimate the standing crop of food organisms available to the salamander larvae. A sampling tube enclosing a surface area of 0.5m^2 was trusted into the pond substratum, the enclosed water column was filtered through 0.05 mm gauze, and the upper 4cm of substratum was sieved through a 1 mm sieve. All macroinvertebrates were collected and preserved in 80% ethanol. The organisms were classified as potential salamander food, i.e. edible or inedible according to previous investigations of salamander larvae gut contents, for details see methods Chapter 1 and Weitere et al. 2004. The edible proportions which composed ca. 99% of the macroinvertebrate community was considered in the following.

To describe and compare the diversity of the macroinvertebrate community, Shannon's diversity index (H') was used. Shannon's diversity calculates from the number of individuals of one group (N) and the number of occurring groups (S). Herein p_i is the proportion of individuals (n_i) belonging to each species.

$$H' = -\sum_i p_i \ln p_i \quad \text{, with } p_i = \frac{n_i}{N} \tag{9}$$

Quantification of insect emergence and prey floating on the water surface

Aquatic insect emergence was quantified using pyramid emergence traps (Davies 1984). The traps consisted of a Styrofoam float and a hood, covering a surface area of 1 m^2. Each trap was equipped with a collecting jar on a top opening of 1cm but without preservation fluid. Both pond halves were each equipped with a single emergence trap and the catching jars were set discontinuously at 30 different dates in three days intervals, throughout the sampling period. The collecting jar was emptied after 24 hours and collected insects were preserved, identified and measured to calculate biomass.

In order to quantify the amount of insects that falls onto the water, all particles larger than 0.5mm were removed from the water surface using a rectangular 30cm hand dip net 24 h before the sampling. For the sampling all invertebrates larger than 0.5 mm were collected from the water surface, preserved in 80% ethanol, identified, measured and biomass was calculated. The sampling was conducted on 5 random dates throughout the investigation period from March till June.

Salamander gut content analyses

For a referential quantification of salamander larvae food spectrum and preferences, the gut contents of 201 larvae from the Ponds P1 (52 ind. in 2011, 33 ind in 2012) and P2 (74 ind. In 2011 and 42 ind. in 2012) were analysed. Therefore a maximum of 15 larvae each month and from each enclosure were preserved in 80% ethanol. Care was taken that the number of removed larvae did not exceed 4% of the total pond population. To avoid a bias of experimental setup the 15 larvae were replaced with larvae of the same size from the backup pond (that held the larvae which were removed from the exclosure). Only the stomach proportion of the digestive tract was considered, all food items within the stomach were classified and measured. The body length or hard part length of all organisms was measured and compared to the environmental samples. Again, body mass was calculated using length dry weight correlations from the literature as described above.

Analyses of stable isotope signatures of salamanders and prey organisms

For a detailed description of the food web structuring and resource usage in relationship to the presence of the salamander top predators, we used stable isotope ratios of 13 C and 15 N composition of potential food resources and consumers on taxonomic level. The isotope sampling was conducted in April and June in both 2011 and 2012. For this, individuals of all main taxa and salamander larvae were sampled and identified. Here, also taxa that were not amongst the salamander food items (specifically Tadpoles and larvae of the northern hawker dragonfly *Aetna cynaea* as well as water striders of the genus *Gerris*) were additionally sampled and included for analysis of changes in food web topology. All organisms were kept in the laboratory on particle free water for 24 h upon freeze-drying. At least 5µg of dry mass was needed for analysis, so individuals were pooled if necessary. Larger individuals were processed individually. Freeze-dried samples were grinded and stored in glass snap cap jars. Between 5 µg and 3 µg dry mass of the resulting powder was precisely weighed and encapsulated in tin foil caps. Delta proportions of the N and C isotopes were measured and analysed by the Stable isotope laboratory of the University Koblenz-Landau in Landau (Delta V Advantage coupled with a ConFlo IV). All data were corrected for isotopic fractionation by adding a trophic discrimination factor of 3.4 ‰ for $\delta^{15}N$ and 0.4 ‰ for $\delta^{13}C$ (Brauns et al. 2012).

3.3 Results

Difference in prey biomass and community composition throughout the season

Mean biomass of food organisms was highest at the time of depositioning in March and April with between 0.25 and 6.57 g DM m^{-2} (Fig. 3.1A) in the enclosure compartment and between 0.02 g DM m^{-2} and 9.38 g DM m^{-2} in the exclosures. During the first month the invertebrate community was mainly composed of larvae of the culicid mosquito *Aedes vexans,* which could account for up to 99% of all macroinvertebrate biomass (Fig. 3.1 A and B). In both setups, the overall invertebrate biomass is drastically reduced by the end of May after the culicid larvae leave the pond (Fig. 3.1 A). As a result, the lowest mean prey biomass was observed in June with less than 0.01 g DM m^{-2} present in all ponds. By late June, the invertebrate biomass slightly increases in both enclosures and exclosure with mean biomass 0.02 versus 0.04 gDM m^{-2}. This increase could be allotted to the appearance of red *Chironomus sp.* larvae to some degree of a second Culicid species in the samples.

The differences between the mean prey biomass contribution in enclosure and exclosure could at no point of time and no site be shown to be significant (Mann-Whitney-U $p>0.05$). Neither was the size distribution of the mean taxonomic groups or the Shannon diversity between the treatments (Mann-Whitney-U $p>0.05$). We could also not detect any species that was present in only one of the treatments. Several larger insect larvae such as Tabanidae and Tipulidae (summarised here as “other dipterans”, Fig. 3.1) occurred only randomly as single finds in both enclosure and exclosure. All predominant species were identical in both setups. The mean Shannon diversity over all dates and all ponds was 0.8 in enclosure versus 0.9 in exclosure and again not significantly different (Mann-Whitney-U, $p>0.05$). For further details on the inter-annual variability in food composition and abundance of the pond invertebrate community see chapter 2.

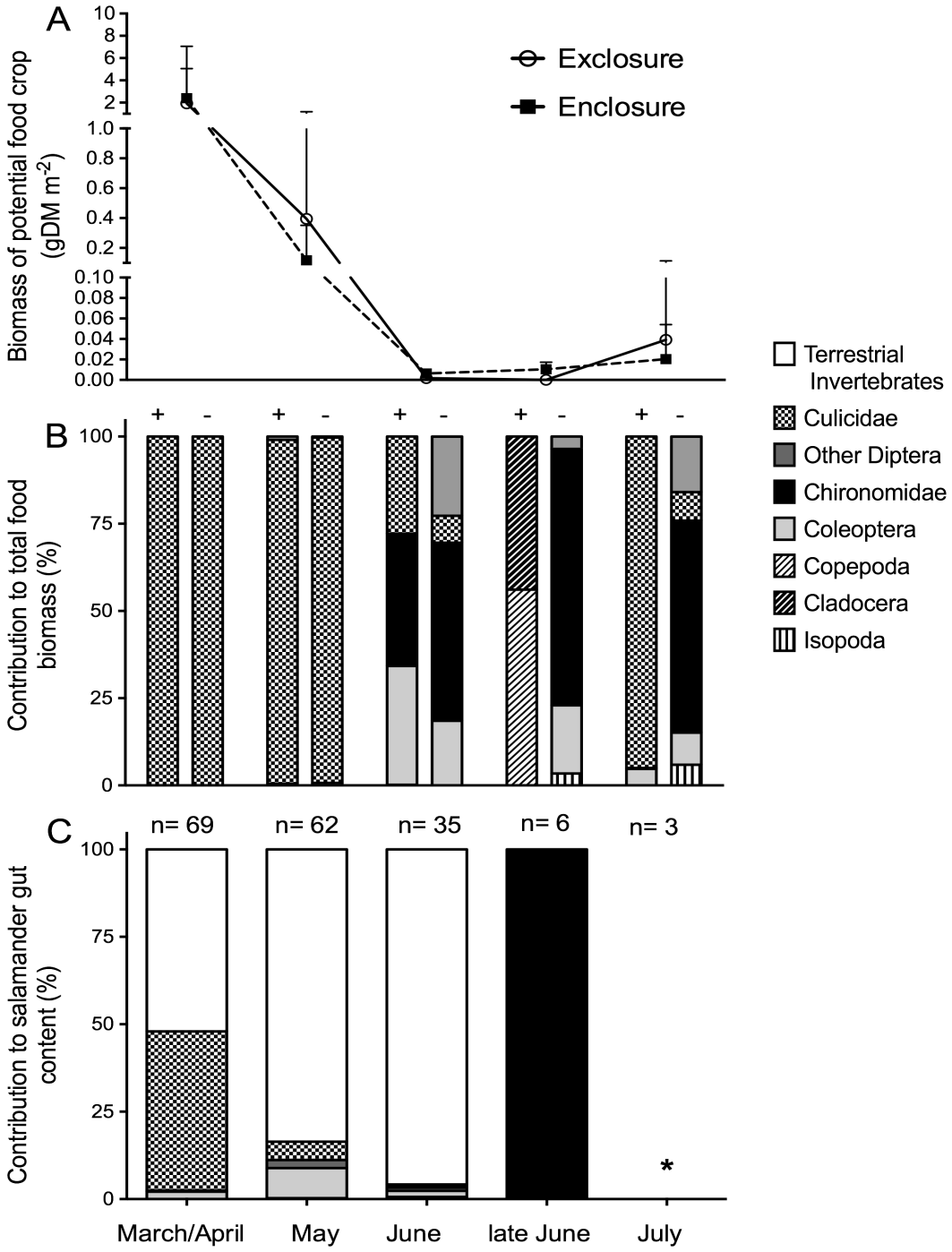

Fig. 3.1: Macroinvertebrate food crop vs. ingested food spectrum. (A) Edible macroinvertebrate biomass in the enclosure (full line) and exclosure (dotted line) area (mean + SE of two sites and two years). (B) Taxonomic composition of potential salamander food organisms as percent of total standing crop biomass in enclosure and exclosure, % of monthly mean of three samples each in two sites of two years. (C) Prey selection: taxonomic composition of invertebrate food in salamander larvae stomach contents (n = number of sampled larvae, % mean of sampled larvae in two ponds and two years). *All sampled larvae without identifiable gut content.

Top predator resource usage and subsidisation of the food web

The macroinvertebrate community was also mirrored in the gut content composition of the salamander larvae. All dominant prey groups were present in the gut contents. The ingestion of culicids during the first month of development was likewise high in both years even though culicid abundance was lower in 2012 compared to 2011 (see chapter 2). In April, culicid larvae made up 45 % of the mean of all ingested food biomass of all salamander larvae. In May this proportion decreased to 6 % as the culicidae became less abundant. Planktonic organisms (Cladocera and Copepoda) only accounted for 0.02 and 0.01 % of the mean gut content biomass. For a detailed consideration of the food selectivity in salamander larvae see chapter 4. In all samples and both years, the ingestion of aquatic food items was heavily subsidised with terrestrial prey such as spiders, caterpillars and adult dipterans (Fig. 3.1C). Large terrestrial items could be found in 14 % of the larvae. In the first month the proportion of terrestrial prey was 52% of the total mean gut content biomass of two years, gradually increasing to up to 96% in late June when the availability of aquatic food biomass was lowest. The proportion of these terrestrial food resources in the gut content biomass increases throughout the season, while the mean contribution of aquatic food biomass to the invertebrate community decreases (for details on prey selectivity in salamanders see chapter 4, results). By the end of June the proportion of chironomid larvae in the guts increased. In July these insects made up the main food item in the salamander guts. Still, in July only six larvae could be sampled for gut analyses altogether due to the advanced metamorphosis of the salamander larvae and the low amount of remaining larvae.

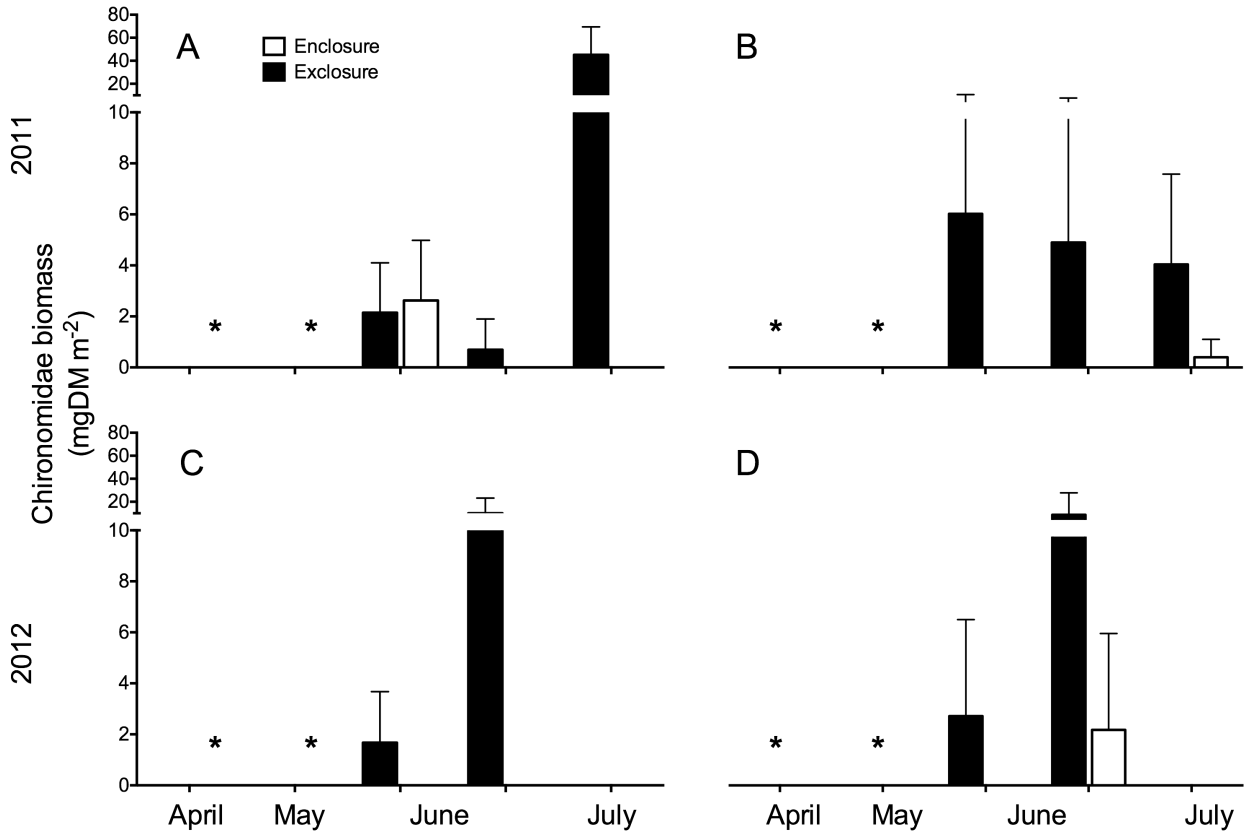

Fig. 3.2: Biomass of larval chironomids in two investigated ponds, comparison between enclosure (white bars) and exclosure (black bars) areas. Mean ± SD in two ponds and two years (A) P1, 2011, (B) P2, 2011 (C) P1,2012 (D) P2, 2012. Empty samples are indicated with *. In 2012 the ponds dried in July.

The influence of salamander larval predation on chironomids

While no significant reduction of the majority of taxonomic groups and the community composition in the enclosures could be observed (see above), there was an influence of the salamander presence on one taxon. While in the exclosure compartment the red *Chironomus sp.* larvae developed biomasses (Fig. 3.2) ranging between 0.0003 g DM m^{-2} (P1, 2012) and up to 0.0731 g DM m^{-2} (P1, 2011), in summer in the enclosure compartments only single chironomid individuals could be noted. The reduction of total chironomid larvae biomass compared to the exclosure treatment in summer was significant for both ponds in 2012 (p=0.041 in P1 and p=0.015 in P2, Mann-Whiney-U-test) and showed the same trend though not significant in.2011 (Fig. 3.3). Furthermore the emergence of chironomid adults from the ponds was also greatly decreased in the salamander enclosure compared to the salamander exclosure. During the peak of chironomid emergence a maximum of 20 individuals m^{-2} d^{-1} could be observed in the

salamander exclosure area as opposed to only 2 during the same period in the enclosure (Fig. 3.3).

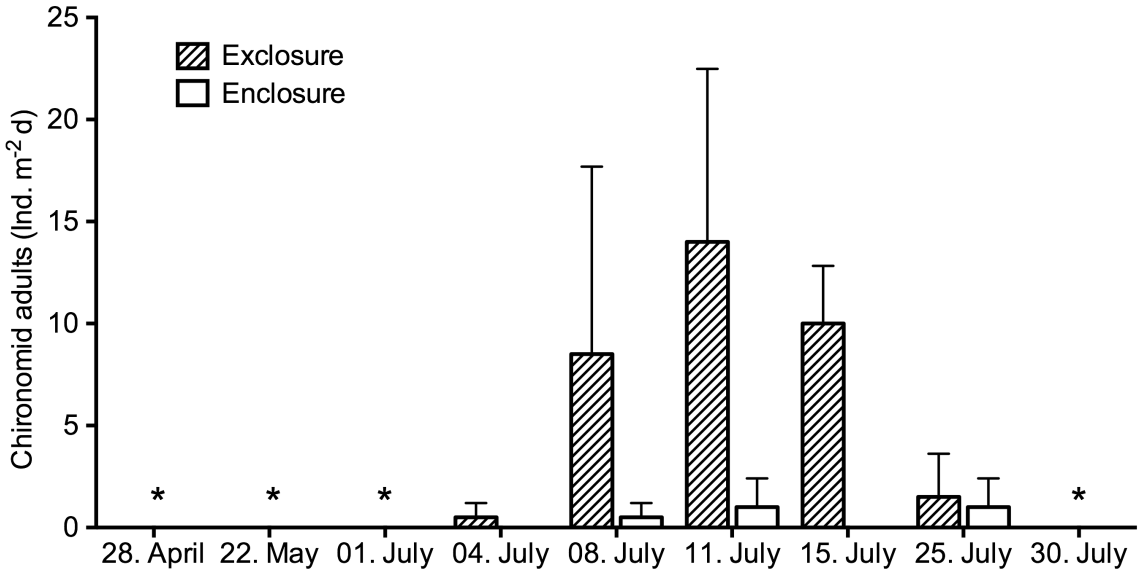

Fig. 3.3: Adult chironomid catch of emergence traps after 24 h exposure. Comparison between enclosure and exclosure in two ponds in 2012 until pond drying (mean and SD). Empty samples are indicated with *.

Salamander larval predation on terrestrial prey

The availability of terrestrial arthropods on the water surface was highly variable at the different dates and the amount of arthropods available from the water surface per day could be as high 0.07 g DM m^{-2} per day but also completely absent on another (Fig. 3.4). Common organisms trapped on the water surface were medium sized moths, spiders and the dipteran genus *Bibio* (which forms large mating swarms over wet soil in spring and dies off after reproduction).

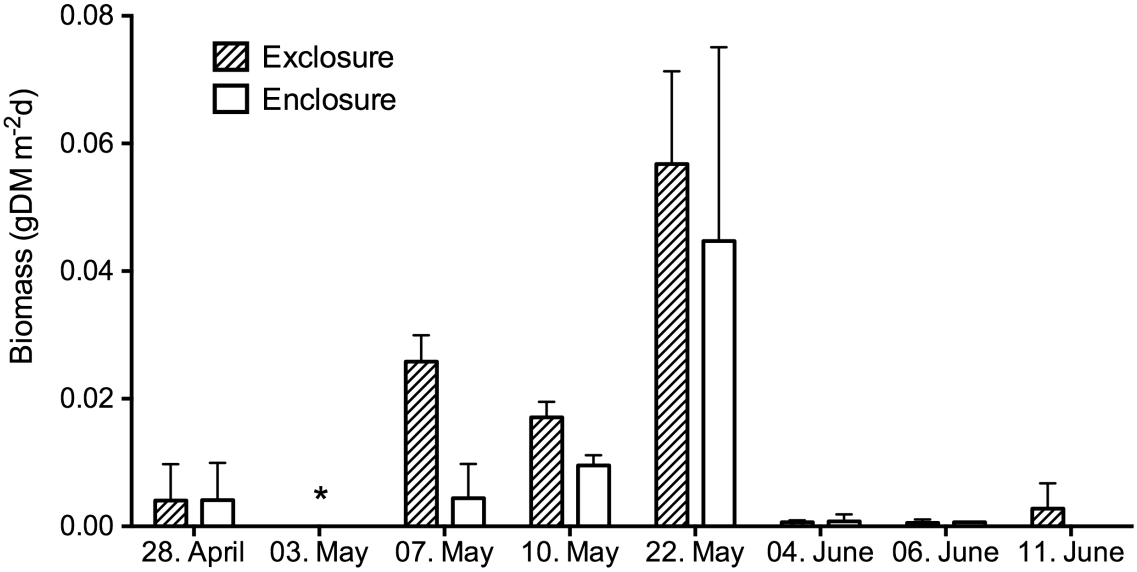

Fig. 3.4: Comparison of amount of arthropods on the water surface in two ponds in 2012. Daily mean ± SD of biomass of arthropods of two pond surfaces at eight random, distinct sampling occasions, distinguished between salamander enclosure and exclosure. 24 h after removal of all floating items. Empty samples are indicated with *.

Salamander density development

Both in 2011 and 2012 the first salamander larvae were found by mid March (20/03/11 and 12/03/12). Initial salamander density at experimental start was quite variable between the years (see also chapter 2) with 37.9 ind. m^{-2} (P1) and 12.3 ind. m^{-2} in 2011 and 143.1 (P1) to 66.2 (P2) in 2012. All initial salamander densities ranged with the measured densities in unmanipulated ponds (compare chapter 4). Salamander biomass remained constant and exceeded the biomass of macroinvertebrates from April onwards and only decreased by the time of metamorphosis (for further details on salamander growth and development see previous chapter 2).

Food web topology and trophic diversity

The ratio of ^{13}C and ^{15}N table isotopes was analysed for all dominant groups of macro-organisms in the pond food web. The seasonal, inter annual and site specific variation lay below the inter sample variation in C and N signatures, thus all data points are considered as replicates here to compare an overall mean and SD for each taxon or functional group. The community is functionally structured from first order consumers with lower $\delta^{15}N$ values to higher trophic levels with higher $\delta^{15}N$ values. Low $\delta^{15}N$ values

were found in the zooplankton, chironomid larvae and tadpoles of the common frog (*Rana temporaria*). In comparison, higher N values were seen in the marsh beetles *Agabus, Hydroporus* and *Aeshna cynaea* larvae. The highest $\delta^{15}N$ values were found in the salamander larvae. The $\delta^{13}C$ values did not show any clear structuring and relating to basal food sources (detritus, leaf-litter, plankton) was not possible (Fig. 3.5).

The individual $\delta^{15}N$ and $\delta^{13}C$ values did also not differ significantly (Mann- Whitney U-test $p>0.05$) between enclosure and exclosure compartment for any taxonomic/ functional group, thus food web topology apparently did not change in relation to salamander presence.

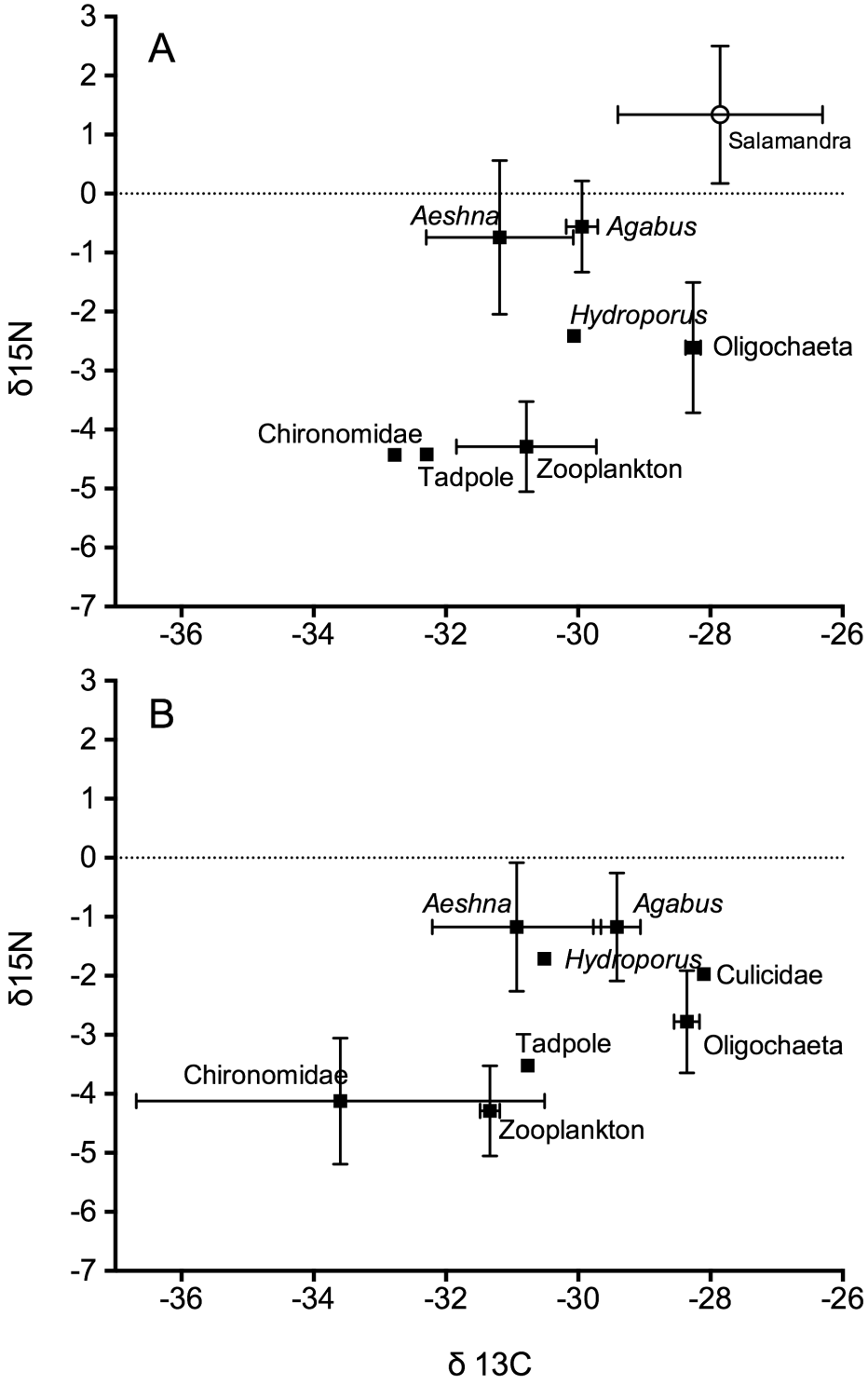

Fig. 3.5: Comparison of ^{13}C and ^{15}N stable isotope ratios of different prey organisms (squares) in (A) enclosures and (B) exclosure area and the salamander larvae (circle). Mean ± SD of two years and two ponds.

3.4 Discussion

Salamander larvae as keystone predators in ephemeral ponds?

Salamander larvae could be demonstrated to act as keystone predators shaping prey communities in perennial and ephemeral ponds in a couple of studies (Holomuzki et al. 1994; Blaustein et al. 1996; Walls and Williams 2001). Our previous estimates based on biomass data and salamander gut content analyses suggested that salamander larvae could also play an essential role in controlling the biotic interactions in pond ecosystems (Chapter 1). As opposed to the initial hypothesis (chapter 1 and resulting hypothesis 1) we find this to be not the case to a large extent for the system investigated here. We could show again, that the salamander larvae can account for an immense biomass that lead to an inverse trophic pyramid. Still, neither the macroinvertebrate composition nor the food web functioning and topology was influenced by the presence or absence of an additional predator species. This is in line with other investigations stating that the intensity of consumptive effects decrease when abiotic stress increases (Chesson and Huntly 1997; Peckarsky et al. 2008; Greig et al. 2013). As we will discuss in chapter 4, ephemeral ponds are highly stressful habitats that require a set of adaptations of every organism to complete the life cycle, a fact that is equally true for predators and prey. The controlling function of abiotic parameters thus like overrules the rather limited effects of the food chain and could to some degree lead to a decoupling of the food web links.

Variable magnitude of trophic effects due to predator and prey lifecycle timing

The strength of interaction between predator and prey species in a food web is usually not equally strong in all trophic links. Commonly one species maintains only few strong trophic links that directly influence each other's abundance and development but various weak links that can aid to stabilise the food web (Huxel and McCann 1998).

The impact of top down pressure by a top predator species is highly depended on the predation susceptibility, the feeding preferences of the predator (e.g. optimal foraging) and the prey species' lifecycle attributes (Abrams and Ginzburg 2000). We could show previously that a good matching of reproductive timing, suitable temperature regime and food organism biomass development is essential for the ecological performance of fire-salamander larvae in ponds but should vice versa influence the magnitude of trophic interactions (chapter 2). We assumed that the consumptive effects of the top predator species would be taxon specific depending on the strength of the food web interactions.

The populations of species that hatch early in the season such as the larvae of the culicid *Aedes vexans* which develop from eggs deposited in the leaf litter in previous years could not be shown to be effected by salamander presence even though they were widely consumed. Yet, the food demand of the salamander larvae in early spring especially at low temperatures is still low and the reduction of *Aedes* larvae might fall below the natural sample variation. *Aedes* biomass in spring moreover is extremely high compared to the developing biomass of other dipterans during the summer. In contrast to the low effect on this abundant prey species a significant effect could be observed on the population development of chironomid larvae that reach their developmental peak by early June and thus in time of highest food demand (due to high temperature and increasing salamander larvae size) and lowest overall aquatic food biomass. Here, the overlap of high food demand and the prey lifecycle create a strong top down link. On the other hand, these play a relatively minor role in the food spectrum of the salamander larvae even though readily consumed, as the population never reaches sufficiently high densities, consequently the bottom up link is relatively weak. Despite the fact that consumptive effects remained restricted to single prey species this had an effect on one integral ecosystem function, the aquatic terrestrial transfer of biomass, which could already be described in chapter 1. It could be shown that by reducing the abundance of chironomid larvae in the ponds, the salamander larvae directly reduced the transfer of adult chironomids to the surrounding riparian habitats. While we assumed this disjunction of one aquatic terrestrial link on the basis of food spectrum analyses previously (chapter 1), it could be observed to be true specifically for Chironomidae here.

Subsidisation of aquatic food web on multiple levels decreases trophic effects within aquatic food web

Ephemeral pond food webs are naturally low in taxonomic diversity. Moreover an overabundance of predatorial taxa often occurs (Williams 2005). In this study we could see that the total macro-invertebrate biomass drops dramatically in summer regardless of top predator presence. The macroinvertebrate fauna is mainly composed of insect larvae with terrestrial or semi-terrestrial imagines that leave the water in summer and thus only partially overlap with the highest food demand of salamander larvae. Both the harsh environmental filter that reduces macroinvertebrate colonisation and persistence

regardless of predator presence and the consequent poor matching between predator and abundant prey in the ephemeral ponds lead to a difficult situation for the predators. We could show in the experimental approach that the persistence of the salamander larvae as top predators in these small ephemeral ponds is dependent on significant amounts of terrestrial subsidisation. In fact, the terrestrial subsidisation of food intake can account for more than 90% of all ingested prey biomass. Presumably it even replaces some of the usual aquatic food resources so that the aquatic community is not depleted to complete extinction.

Especially in the late season the larvae spend significant time close to the water surface probably to increase oxygen supply and consume terrestrial prey. Benthic organisms can escape predation in the sediment if they can withstand low oxygen conditions (like red chironomidae). Also optimal foraging choices would relieve small plankton such as copepods and cladocerans from predation in favour of more profitable terrestrial items. (See also discussion on food preference in chapter 4).

The third hypothesis that input of terrestrial invertebrates can drive the trophic cascade within the aquatic system and stabilise higher positions in the food web could be confirmed in this experiment. In chapter 1 we found that terrestrial subsidisation can account for a remarkable proportion of ingested food biomass. This relationship has so far been noticed mainly for riverine and large lentic systems where fish production and population development is often subsidised by terrestrial resources (Polis et al. 1997; Mehner et al. 2005). Small aquatic habitats share a larger boundary line with the surrounding habitats (i.e. the forest). If the surrounding habitat is more productive than the aquatic system the available amount of terrestrial subsidies can be large and the matter exchange can become a crucial subsidisation of the aquatic food web (Polis et al. 1997; Mehner et al. 2005). While the primary consumer largely rely on immense amounts of leaf litter and terrestrial plant detritus, higher-level consumers feed on terrestrial invertebrates that commonly fall onto the water surface. Some pond species such as pond skaters (e.g. Gerridae) and water boatmen (Notonectidae) are known to largely rely on this food resource especially if developmental time is limited (Pfenning et al. 2007). Here we could demonstrate the importance of terrestrial subsidisation for persistence and growth of an amphibian species in the ponds allowing for a coexistence of various predator species and low amounts of potential prey.

The high amount of subsidisation in the higher trophic levels could explain why we cannot see any change in food web topology as assumed earlier (hypothesis 4). Shifts in food web properties such as increasing omnivory and thus shifts in diet composition of other species occur if the community is released from a top predator species and thus predation pressure or competition is reduced (Walls and Williams 2001; Verburg et al. 2007). In the investigated ponds the different components of the food web are decoupled by the terrestrial subsidisation and so the predation pressure on aquatic organisms is not as high as would be expected.

"Terrestrialisation" of pond food webs and salamander larval development

Due to the limited time to complete their development in the pond and increasing resource limitation, the pond breeding ecotypes of the fire salamander reduce their stay in water by increased size at birth and a reduced metamorphosis threshold (Weitere et al. 2004). Here we could demonstrate that also the food intake is largely decoupled from aquatic resources, even greater than assumed previously (chapter 1). While at times of high food availability the terrestrial subsidisation was only 50% of all gut content biomass, its proportion raises to more than 95% in summer when other food items are scarce (Fig. 3.1). Terrestrial invertebrates could be demonstrated to be available at all times throughout the season (Fig. 3.3) and the salamander larvae rely on this resource. Even though the larval development takes place in an aquatic environment, we could show here that the evolution to reproduce in ephemeral ponds in Western Europe leads to an increased dependence of terrestrial residues. This might in turn also lead to an increased "terrestrialisation" of the life cycle and development, a trend that is seen in other fire salamander (sub-) species. Those often show altered reproduction modes such as the complete retention of larval stages in the mother uterus and birth of fully developed metamorphs (juvipary) (Veith et al. 1998).

Conclusion

The range of potential salamander larval pond habitats is very broad from very short lived to nearly perennial systems and we would predict different outcomes and functioning in any subset of parameters. Here the implications of salamander presence in highly unstable ephemeral forest ponds were observed. In conclusion it can confirm earlier findings of chapter 1 that the pond reproducing fire salamander larvae are a

highly abundant predator in the pond ecosystems and lead to an inverse trophic pyramid. Unlike expected in hypothesis 1 no general strong top down control of prey organisms or a drastic alteration of the food web topology could be observe as a result of salamander presence. On the other hand consumptive effects could occur taxon specifically as mentioned in hypothesis 2. As assumed by the third hypothesis salamander larvae persistence relied on a very high degree of substitution by terrestrial resources. Altogether this implies that the ephemeral pond food webs including the pond ecotype salamander larvae exist as terrestrial periphery rather than independent aquatic systems in terms of resource availability.

4. Chapter

New home, new life: Population development, performance and selective pressures of pond versus stream breeding salamander larvae

4.1 Introduction

Evolutionary shifts in habitat choice of a species can be caused by a variety of factors. Commonly competition and resulting niche partitioning are argued to be key factors, driving populations to adapt to novel environments (Schoener 1974). Also alterations in the environment such as climate change or anthropogenic habitat manipulation can force a species to acquire new habitats (Schluter 2001; Wiens 2004). In the light of ongoing habitat destruction and fragmentation, the capability of adapting to new environments is beneficial to colonise and re-colonise habitats (Walther et al. 2002). Nevertheless, colonising new habitat types is often connected with a setting of new environmental influences and restrictions and consequently will require new adaptations in ecological performance and phenology.

The fire salamander (group of several species within the genus *Salamandra*) is far spread throughout Europe and the Middle East. Today several species with various subspecies are distinguished, that inhabit deciduous forests to semiarid deserts (Blaustein et al. 1996; Steinfartz et al. 2000; Thiesmeier 2004). Moreover, fire salamander populations show a high adaptability to various environmental conditions in both the larval and adult phase. The European fire salamander (*Salamandra salamandra*) is a typical inhabitant of deciduous broadleaf forests in mountainous areas of Central and Western Europe. Most populations deposit their larvae in first order streams where fish are absent (Thiesmeier 2004) but some have also adapted to utilise various kinds of small lentic water bodies such as underground springs, drainage ditches, wheel ruts and natural water-filled depressions (Thiesmeier 2004; Weitere et al. 2004; Manenti et al. 2009). In some cases these pond breeders could be demonstrated to be genetically separated from populations breeding in streams in striking distance (Steinfartz et al. 2007b). This kind of local adaptations happened quite recently after the postglacial re-colonisation of Western Europe by the salamanders (Weitere et al. 2004;

Steinfartz et al. 2007b). First order streams in temperate regions are typically perennial and provide a stable and relatively benign environment. in the short fast flowing streams the temperature regime is constant, the high mixing of the water column provides a high oxygen content and harmful decomposition residues such as ammonium rarely build up (Lampert and Sommer 2007). Salamander larval densities in the streams usually remain low as the larvae can disperse downstream (Thiesmeier and Schuhmacher 1990).

Small pond environments on the other hand are highly unstable systems. In the previous chapters various environmental restrictions for the pond breeding ecotype have been assigned: Salamander larvae in ponds face a limited time to metamorphosis due to short hydroperiods (Weitere et al. 2004). The risk of mortality due to oxygen depletion, overheating or accumulation of harmful decomposition of the high amounts of leaf litter is high (see chapter 1). The high inter annual variability in parameters such as temperature regime, hydroperiod und food availability demands a high flexibility in the salamander life cycle (chapter 2). Persisting high salamander larval densities result in overexploitation of aquatic food resources (chapter 1 and 3).

The larval phase of three to six month constitutes only a small fraction of the fire salamander's life of up to 20 years (Thiesmeier 2004). Yet, it crucially determines the reproductive success and the selective pressures on phenological adaptations in this life stage should be very pronounced. This chapter aims at comparing the environmental settings of ponds and stream habitats and assess their relevance for the ecological performance of the fire salamander larvae. The potential selective factors acting upon salamander development and ecological performance during the early life in stream and pond habitats will be analysed in order to identify potential drivers of the genetic split between pond and stream ecotypes.

4.2 Material and Methods

Location and study site description

The studied sites are located in the Kottenforst as described in details in the previous chapters 2 and 3. The area hast various small pond habitats of which many anthropogenic origin (e.g. second World War bomb craters, roadside ditches and machine tracks). Groundwater-fed springs are also common in this area and form first

order streams on the sides of the topographical plateau. All first order streams are between 700 m (S3) and ca 1099 m (S1) in free flowing length (Table 4.1). Only the uninterrupted free flowing surface parts proximal to the spring are considered here. The free flowing stretches end either in systems of sewage canals or larger second order streams which both are unsuitable for salamander survival. All streams in this investigation are typical lowland headwater streams with a stream channel in slightly-sandy soil and large amounts of detritus such as wood debris and leaf litter. Previous analyses demonstrate that both, stagnant ponds and first order streams are frequented equally for reproduction by fire salamanders but populations from both habitats show distinct genetic differences (Weitere et al. 2004).

The present study investigated four stream sites and six pond sites (Table 4.1). Of these, four pond sites (P1- P4) have already been considered in chapter 2 and 3. P1 and P2 were separated in en- and exclosure segments (see chapter 3 methods) with and without salamander presence. In this investigation we only considered the enclosure segments. Here we compared data on the salamander larvae populations and macroinvertebrate communities during the salamander larvae season between March and July in the year 2011. Of the six ponds, the macroinvertebrate community of four has been resampled in the year 2012. Chapter 2 addresses the data on the inter-annual variability of the macroinvertebrate community and salamander performance in detail.

Abiotic parameters

All monitored sites were equipped with EBI T20 (EBRO) temperature loggers recording the temperature at 10 cm (ponds) or 2 cm (streams) water depth in 2 h intervals. Furthermore the water level of the ponds was measured weekly using an installed measuring staff. Hydrochemical parameters such as ammonium, pH, and oxygen were also monitored but either found not to be distinctly different or below harmful concentrations for aquatic organisms (Table 4.1). For a more detailed consideration of the influence of abiotic parameters on larval survival see chapter 1. No mass mortalities associated with any of these parameters could be recorded during the year 2011.

Macroinvertebrate sampling

Benthic macroinvertebrates from ponds and streams and zooplankton from the ponds were sampled monthly with three replicates each in order to estimate the standing crop of food organisms available to the salamander larvae. For details on the sampling of macroinvertebrates and estimation of food crop biomass see respective methods of chapter 1 and 2. Only edible proportions of macroinvertebrates were considered in the analysis (see chapter 1)

Larval deposition, growth and metamorphosis

The initial salamander larval densities in the ponds were assessed by the recapture method (Youngs and Robson 1978). For details see methods of chapter 1. Date of first larvipositoning was observed during four years (2010- 2013). During 2011 all captured larvae were weighed, measured and marked by digit amputation. Afterwards they were released back into the pond immediately and recapturing was conducted 24 h later. To screen for population fluctuations and estimate the later population size we used time per unit afford sampling and sampled larvae for ten minutes using a standardised triangular amphibian scoop (Schlüpmann et al. 1995). All captured larvae were weighed and measured again to calculate size development. Due to low recapture probability, stream larvae density was estimated each month by counting all larvae per one m^2 at sight. Therefore we assessed the density of ten randomly selected segments in the first 100m from the spring of each stream, each consisting of one m length. The segments were enclosed with a large scoop net downstream and all larvae that could be found upstream were captured and counted. Furthermore the entire streams were screened for recaptures once between the 25th and 29th of June. All recaptured marked larvae were assigned to their marking point and the drift distance since the last capture was measured.

Larvae at all pond sites were recaptured frequently every three to four weeks to quantify growth increment and population development. For the ponds the majority of larvae were sorted into birth/age cohorts and marked accordingly, so we could note and calculate cohort growth. For methodological details see methods of chapter 3. For the streams we calculated the mean size of all captured larvae each three weeks.

All metamorphs in the ponds were controlled by enclosing amphibian fences (obtained from Ehlert & Partner GbR) and weighing of fresh metamorphs. Fencing was established

after the reproduction peaks when no reproduction could be observed anymore despite suitable rain for more than four weeks and the initial population density was within the range of densities observed in un-fenced ponds. In the streams fence capturing was not possible due to the large area that had to be covered. The streams were screened for larvae, which showed definite signs of metamorphosis (i.e. reduced gills, changed head proportions) at every sampling occasion. Those were captured and raised in the laboratory for 24 h until metamorphosis was complete (see methods chapter 1). This procedure allows an estimate of the average metamorphosis size but, unfortunately, did not allow for a detailed investigation of metamorphosis success in the streams since a high proportion of metamorphs was probably overlooked.

Quantification of salamander gut contents

For a referential quantification of salamander larvae food spectrum and preferences, the gut contents of 166 pond (126 from the ponds P1 and P2 in 2011) - and 117 stream-larvae were analysed. Therefore a maximum of 15 larvae each month, less in the end of the season, were preserved in 80 % ethanol. Care was taken that the number of removed larvae did not exceed 4% of the population size. We considered only the stomach proportion of the digestive tract, in which all food items were classified and measured. For details on stomach content analyses see respective methods of chapter 1 and 3.

To analyse the selectivity of salamander feeding behaviour, the electivity index after Jacobs (1974) was calculated. This index (D) compares the relative contribution of each food taxon (k) in the environmental sample biomass (P_k) to the contribution of the same taxon in the gut content biomass (R_k).

$$D = (R_k - P_k)/(R_k + P_k - 2R_kP_k) \quad (10)$$

The index can have values from -1 for a complete avoidance to +1 for a clear preference. A value of 0 indicates that there is neither preference nor avoidance of a specific group. Here the mean electivity of all month from March to June is given. The July sampling was excluded because it did not contain the necessary comparable amount of 15 gut content samples per site.

4.3 Results

Temperature framework

The temperature regime in the groundwater fed headwater streams was fairly constant. During the early year the general mean water temperature was 9.7°C and the temperature never fell below 8.5°C. Peak temperatures were noted in July and August at 12.7°C and highest mean temperature of 12.3°C in July. Variation between the sites was less than 0.5°C at most times (Fig. 4.1A). Nonetheless, temperature values above 11°C where rare in the streams. The pond temperature regime was more variable both daily and within the season. Yet, the inter site variability in temperature regime of all investigated ponds was low. While starting below 5°C in March during larvipositioning, the water could already heat up to more than 10°C by early April with temperature fluctuations of more than 5°C within 24 h (Fig 1D). Water temperatures in the ponds were constantly above 10°C from May onwards, with extremes of more than 20°C in July. While the mean temperature in April during early larval development was only 8.5°C in the pond, it was 13.5°C during the peak of metamorphosis in June (Fig. 4.1D). The minimal temperature threshold for notable growth in salamander larvae is 6.47°C (See Appendix 1). Stream living salamander thus never experienced temperatures below this threshold while pond larvae were subjected to low temperatures commonly early in the season.

Table 4.1: Abiotic and salamander larval population parameters in all investigated sites in 2011. In P4 initial and P5 metamorphosing larval density was not estimated (n.e.) in 2011.

Site	Site type	Length (m) resp. Area (m^2)	pH	Temperature range during larval phase (°C)	NH4+ ($mg\ l^{-1}$)	Intitial larval density ($ind\ m^{-2}$)	Larval density at first metamorphosis ($ind\ m^{-2}$)	Hydroperiod (in 2011)
S1	Stream	1099	7.5	9.2- 12.8	Max 0.03	6.1	0.2	perennial
S2	Stream	3000 (ca 700 free flowing)	8.0	9.8- 12.3	Max 0.03	2.7	0.1	perennial
S3	Stream	700	7.5	9.8- 12.0	Max 0.03	3.1	1.0	perennial
S4	Stream	2320 (ca 500 free flowing)	7.0	10 - 14	Max 0.03	2.3	0.01	perennial
P1[a, b]	Bomb crater pond	12.6	7.0	5.1- 18.2	1.7-2.8	37.9	54	Ephemeral till July
P2[a, b]	Bomb crater pond	14.1	7.1	5.1- 18.2	1.7-2.9	12.2	114	Ephemeral till July
P3[a]	Bomb crater pond	13.8	6.9	5.1- 18.2	1.2-4.3	3.6	0[c]	Ephemeral till June
P4[a]	Bomb crater Pond	15.0	7.0	5.1- 18.2	1.7-2.9	n.e.	0[c]	Ephemeral till late May
P5	Roadside drainage ditch pond	2.9	7.0	5.0 – 22.0	4.2-4.9	21	n.e.	Ephemeral till June
P6	Natural depression pond	22	6.7	5.0- 18.2	3.0- 8.0	9	2.4	perennial

[a] sites that have been considered in chapter 2,
[b] sites considered in chapter 3,
[c] ponds dried before metamorphosis

Food availability in both habitats

In the stream habitats, the biomass of macroinvertebrates was high throughout the year. No significant change in macrozoobenthos abundance could be noted, though the general available biomass decreased towards the summer. The mean biomass ranged from 2.03 ±1.66 gDM m^{-2} in May to 0.72 ±0.66 gDM m^{-2} in July (Fig. 4.1B). Mean salamander larvae biomass was 0.06 ± 0.04 gDM m^{-2} after the reproduction peaks and decreased to 0.001 ± 0.0401 gDM m^{-2} before metamorphosis in late May. While the conditions in the streams were relative constant, the ponds showed strong inter-annual fluctuations in macroinvertebrate availability and salamander larvae abundance (see more chapter 2 for specific details). Highest amount of macrozoobenthos was observed in April with 4.06 gDM m^{-2}. The lowest amount was noted in July with only 0.59 gDM m^{-2}. In the stream sites the salamander dry mass never exceeded more than 10 % of the available food organism biomass (Fig. 4.1B).

In the ponds, the yearly macroinvertebrate dry mass was low compared to the stream sites. Only in April at the time of highest abundance, the pond macroinvertebrate mass reached 0.81 gDM m^{-2} in the site P2 and a mean biomass of 0.59 ± 0.35 gDM m^{-2} on account of large amounts of culicid larvae could be noted (Fig 1 E, see also chapter 2). The macroinvertebrate mass dropped to a mean of 5.64 10^{-7} ± 1.21 10^{-7} gDM m^{-2} in late June as all dominant insect taxa emerged from the pond (for a detailed investigation on the macroinvertebrate development in the ponds, see chapter 3). The salamander biomass equals the amount of macroinvertebrate biomass in April and does not change much throughout the season until metamorphosis starts, thus surmounting the amount of available macroinvertebrates from March onwards. Highest salamander biomass found in the ponds was 1.61 gDM m^{-2}. Mean Salamander biomass ranged between 0.97 gDM m^{-2} in May and 0.34 gDM m^{-2} in June before the majority of larvae metamorphosed (Fig. 4.1E).

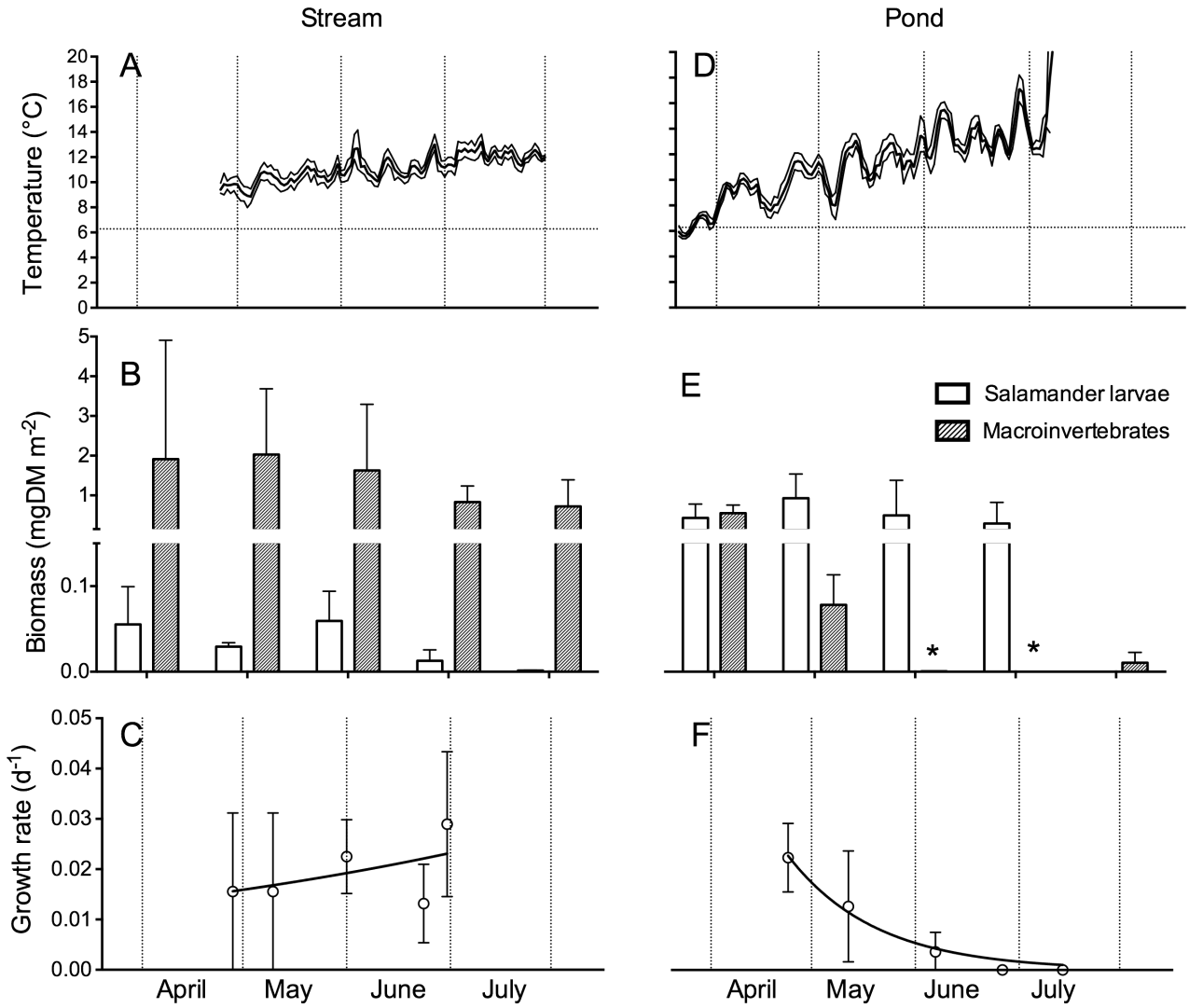

Fig. 4.1: Comparison of environmental parameters and growth of salamander larvae in ponds and streams. (A, D) Temperature development in streams (A) and ponds (D) in 2011 (Daily mean temperature ± daily min. max). The horizontal dotted line represents minimal temperature for growth (6.47°C). (B, E) Available food mass (macroinvertebrates, white bars) versus salamander larvae biomass (grey bars) in streams (B) and ponds (E). Dry mass values below 0.001 g are indicated with *. Data are represented as means of six ponds and four streams ± SE. (C, F) Development of mean growth rates of salamander larvae in streams (C) and ponds (F).

Macroinvertebrate composition vs. food selectivity

The macroinvertebrate community in the different streams was similar and variation between different sampling points was also low. The macrozoobenthos community in all streams was strongly dominated by the amphipod *Gammarus pulex,* which could be present in high densities in the decaying leaf litter. The species could comprise between 42.6% of all benthic biomass in the streams in July and 66.2% in April (Fig. 4.2A). Furthermore especially endobenthic organisms such as the small clam *Pisidium* or endobenthic dipterans such as *Ptychoptera sp*. and chironomids were common. Ptychoptera were most common in the streams S1 and S4 with between 16.3 and 43.3% of all macroinvertebrate biomass. Endobenthic invertebrates were mostly represented by Trichoptera and small Plecoptera species such as *Leuctra sp.*. Amongst the Trichoptera it was distinguished between caddis baring forms such as *Anabolia* and *Sericostoma* and the free living *Plectocnemia.* Other free-living Trichoptera were rare in all sites as well as other hard substrate associated invertebrates such as Ephemeroptera (Fig. 4.2A).

The ponds generally contained a much lower number of macroinvertebrate species. Also the communities showed a high inter-annual variability as described in chapter 2. Culicid larvae dominated the pond invertebrate community in early spring 2011 and could account for 99 % of all organism biomass in that year. They emerged by early May resulting in a strong reduction of macroinvertebrate biomass at that time. Small plankton crustaceans such as cladocerans and copepods remained. Furthermore, the larvae of beetles of the family Scirtidae and the adults of *Hydroporus* and *Agabus sp.* as well as some semi-aquatic Oligochaeta were occasionally abundant in the shallow water and mud (Fig. 4.2B). Furthermore, the larvae of red chironomids could occur in some ponds in small numbers, but were quickly consumed by the salamander larvae. See chapter 3, Fig. 4.1 for macroinvertebrate community development in the ponds.

Feeding behaviour of salamander larvae was largely unselective in both ponds and streams. All organism from the environmental samplings that could be classified as consumable due to size and shape (see chapter 1) could also be found in the salamander stomachs (Fig. 4.2C). In all streams *Gammarus pulex* was amongst the most important food item in the guts with a mean of 91.9% of all gut content biomass in July. The mean Jacobs electivity index was -0.1 for *Gammarus*, consequently only slightly less well represented in the gut compared to the environmental samples. Only in may the mean

contribution of *Gammarus* to the gut content was comparably low with only 13.5% of all gut content biomass. Generally preferred food items were Plecoptera larvae (mean electivity of 0.9), beetle larvae (mean electivity: 0.8) and the caseless caddisfly *Plectocnemia* (mean electivity of 0.4. *Plectrocnemia* accounted for up to 27.8 % of the mean gut content biomass in May. Case making Trichoptera were generally avoided (electivity: -0.9). The same was true for endobenthic prey such as *Ptychoptera sp.* and *Pisidium,* which were not consumed despite their occurrence in the streams (electivity: -1) (Fig. 4.2B, Table 4.2).

In the ponds the preferred prey in spring was the abundant larvae of *Aedes* which could account for a mean of 45% of all gut content biomass. It contributed to the macroinvertebrate community of 99% during the same time. Later in the season the main aquatic food items were cladocerans and Scirtidae larvae as well as chironomid larvae. Anyhow, aquatic food accounted for only a small proportion of maximal 47.6% of the mean food spectrum found in the gut contents of pond salamander larvae which made the calculation of Jacobs electivity for the food taxa highly biased (Table 4.3). We saw earlier (chapter 3), that the aquatic food spectrum is greatly subsidised by the intake of terrestrial prey, which increases as the amount of aquatic prey decreases. With more gut content samples and more ponds this relationship becomes even more distinct. In the ponds the amount of subsidisation could reach 87.9% of the total gut content biomass during the time of lowest aquatic food availability in late June. In the ponds the mean relative amount of terrestrial subsidisation of food intake increased throughout the year (Fig. 4.2D). The majority of larvae consumed at least single terrestrial food items. Overall, the most common terrestrial food items were collembolans, caterpillars and spiders. In the streams terrestrial subsidisation comprises only a small percentage of the food intake but nonetheless was observable. A maximum 7.6% of the gut content was of terrestrial origin (Fig. 4.2D). Flies and terrestrial beetles were most predominant here.

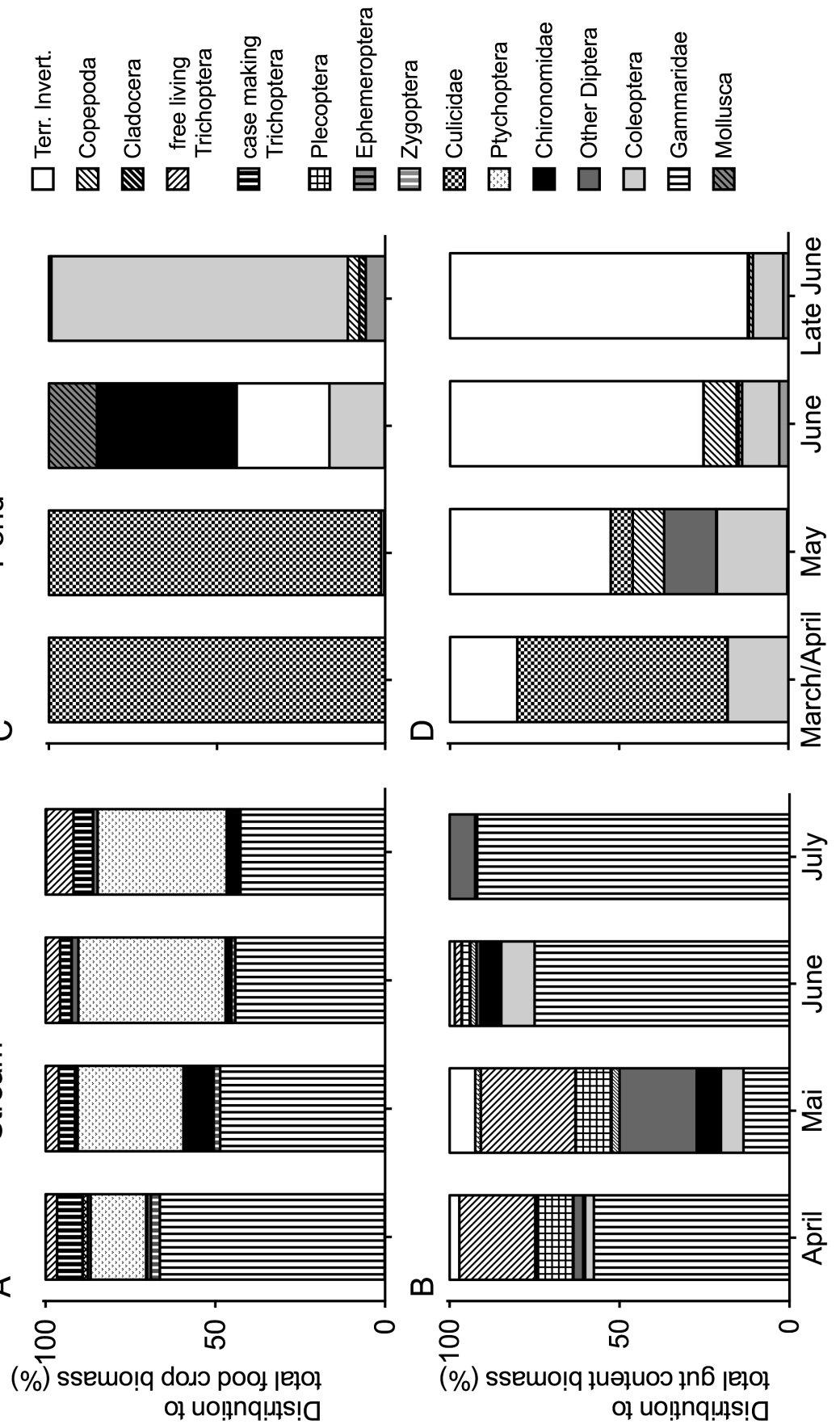

Fig. 4.2: Comparison of (A, B) gut macroinvertebrate community and(C, D) gut content composition of salamander larvae in (A, C) streams and (B, D) ponds (mean of six ponds and four stream sites).

Table 4.2: Jacob's electivity indices for major groups of aquatic food organism in salamander larval gut biomass. Mean of all three sampling occasions between March and June.

Taxon	Stream	Pond
Cladocera	Not present	0.2
Copepoda	Not present	0.3
Ostracoda	Not present	0.4
Gammaridae	-0.1	Not present
Coleoptera	0.8	0.3
Culicidae	Not present	-1
Chironomidae	0.2	-0.3
Ptychoptera	-1	Not present
Other Diptera	0.4	0.8
Caddis bearing Trichopter	-0.9	Not present
Free living Trichoptera (*Plectocnemia*)	0.4	Not present
Ephemeroptera	0.3	Not present
Plecoptera	0.9	Not present
Pisidium	-0.4	Not present

Larviposition timing

Inter-annual variation in the larvipositioning timing in the pond breeding salamander females was high with the date of first observe larvipositioning varying up to 50 days between different years. In comparison, the date of first larvipositioning in streams differed less than 23 days between the years. In all observed years, larvipositioning in the ponds occurred earlier than in the streams. Larvipositioning in the ponds occurred at the earliest on the 20th of February in 2010 und lasted till the end of April. In the streams the first larvae were observed on 28th of March in 2010 and new born larvae could be noted at least till mid June (Table 4.3). The maximal difference between the larvipositioning dates of stream and pond larvae was 36 days in the years 2010 and 2012. The minimal difference between pond and stream reproduction was seen in 2013. In this year temperatures remained below 10°C throughout March and the ponds were covered with ice. Larvipositioning in the ponds only started 10 days before the streams. In all years the first newborns could be observed in all streams synchronously after the first larvipositioning night. In the ponds the time of larval birth could vary remarkably between the sites but all investigated sites here were colonised on the same night.

Table 4.3: Comparison of first observed larvipositioning dates in all ponds and streams in four years.

Year	Pond	Stream	Time difference between pond and stream reproduction (d)
2010	20/02	28/03	36
2011	12/03	15/04	34
2012	05/03	10/04	36
2013	10/04	20/04	10

Salamander larvae density development and drift distance

In the streams the initial larval density ranged between 2.3 (S4) and a maximum of 8.6 ind. m^{-2} (S1) with a mean of 4.1 ind. m^{-2} (Table 4.1). Initial salamander larval density in ponds was always significantly higher than in streams (Man Whitney-U test p= 0.004). The density was reduced to between 1.6 and 2.5 individuals m^{-2} in all streams within a month. When the larvae started to metamorphose in June the larval density was as low as 0.02 to 0.05 individuals m^{-2} (Fig. 4.3A). In a similar manner, the recapture rate amongst marked larvae in the streams decreased by mid to late May about one month after birth. In total 583 stream larvae were marked in all streams between the 20th and 28th of April. Of these a total of 67 larvae were recaptured once (10,3% of all marked larvae). Recapture rate was low in all streams, ranging from 4.8 in S4 to 21.9 % in S2. Within the first month after marking in April none of the recaptured larvae had moved further than 50 m downstream from the release point. Yet, only eight larvae could be recaptured after the end of March. Of these, four larvae were found at the same point as during the last capture (50m within the range of larvipositioning) 70 days after marking (20th April to 29th of June). Two larvae in the stream S2 could be recovered 305 m and 368 m downstream from the previous recapture point, 42 days after the last recapture (17th of May to 29th of June). The first reduction of larval densities and the disappearance of marked individuals coincided with two major rain events of 1 and 2 mm precipitation per night on the 11th and 13h March. During the larval period 2011 only 5 rain events of more than 1mm per 10 minutes could be noted (personal communication Daniel Geller)
Initial larval density in the ponds ranged between 3.6 (P3) and 37.9 ind. m^{-2} (P1) with a mean of 16.7 ind. m^{-2} (Table 4.1). Unlike in the streams, the larval density does not decrease throughout the season (Fig. 4.3B). Recapture probability was high with individuals of every cohort captured with every sampling.

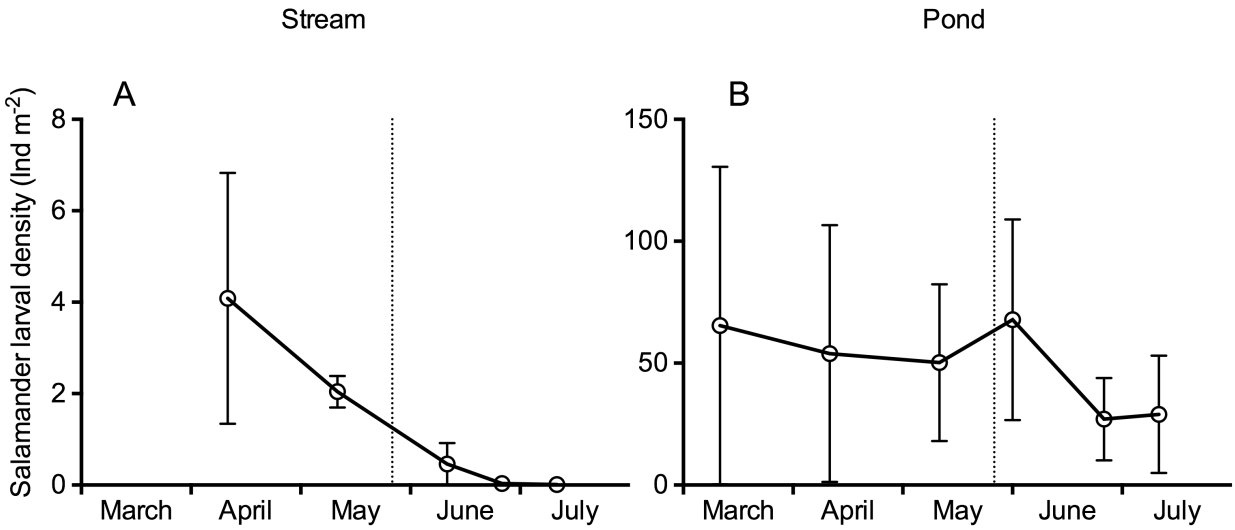

Fig. 4.3: Development of fire salamander larval density in (A) streams and (B) ponds (mean ± SD of six pond and four stream sites). The dotted vertical line indicates time of first observed metamorphs. Note the different scaling of the y-axis.

Salamander larval growth patterns

The growth patterns of salamander larvae in ponds and streams differed remarkably. In the streams the growth rates of the salamander larvae increased with the time. In April at temperatures of roughly 10°C the mean growth rate was 0.016 d^{-1}. It increased until the end of the season, with growth rates of 0.029 d^{-1} at temperatures of 12.3 °C (Fig. 4.4). In contrast, the growth rates in the ponds decreased drastically throughout the season. The highest growth rates were 0.023 d^{-1} at temperatures of 9.8 °C in April (Fig. 4.4). In the streams growth rates were close to the theoretically maximum achievable growth rate for the specific interval temperature if food is not limited (compare Fig 3, and Appendix I). The pond salamander larvae show maximum possible growth only during the first period after birth in April, in the later season the growth rates drop drastically below the possible growth (Fig. 4.4).

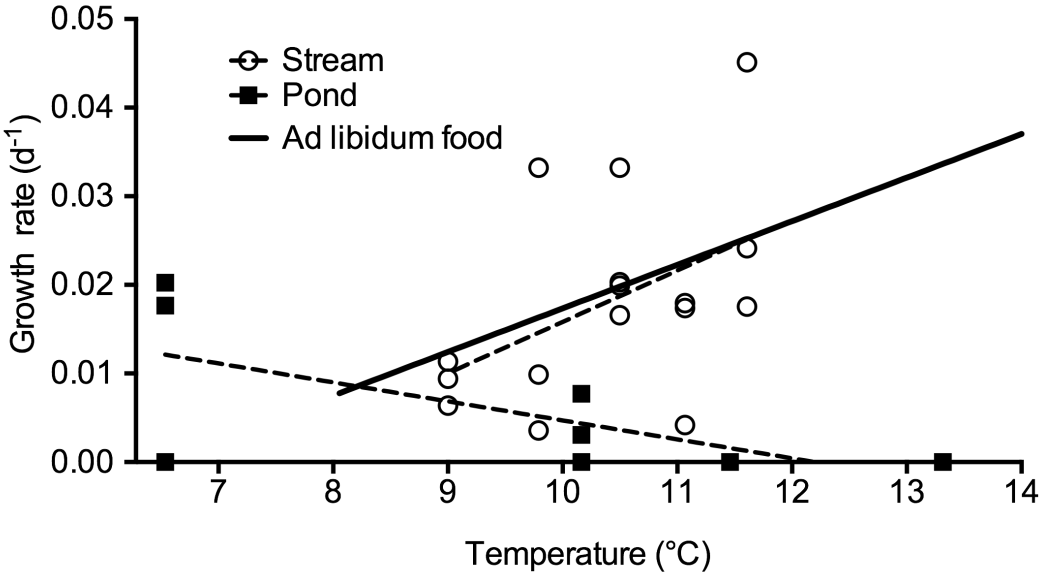

Fig. 4. 4: Relationship between mean growth rate and mean temperature in ponds (black squares) and streams (empty circles) with corresponding regression lines (dotted lines) in comparison to ideal growth rate (solid black line, see also appendix 1) of larvae raised under ad libidum food.

Metamorphosis performance

Salamander metamorphosis was only monitored in 2011. In this year the first metamorphs in the streams could be found on the 29th of June. Thus, the first stream metamorphs left the water 37 days after the first metamorphs in the ponds, which could already be observed on the 22th of May. In 2011 only in two (P1 and P2, see also chapter 2) of the six ponds the larvae survived to metamorphosis, the other sites dried before metamorphosis was completed. The four observed stream metamorphs were as heavy (weight range of 0.46 - 1.04 g fresh mass) after metamorphosis as the 14 observed pond metamorphs (weight range of 0.4– 1.0 g fresh mass) but the low numbers could not be tested for statistical significance.

4.4 Discussion

It was shown previously that pond and stream reproducing salamanders in the Kottenforst area show a certain degree of genetic segregation despite a very small scaled sympatric occurrence (Steinfartz et al. 2007b). The two ecotypes display some reproductive isolation (Steinfartz and Caspers 2011) which could be a result of the need

for differently matched reproductive timing or growth patterns. These factors indicate the first stages of sympatric speciation in this species. In the previous chapters we largely considered the new ecological functioning of pond reproducing salamanders for their habitats (chapter 1 and 3) as well as considered the phenological strategies and constraints of pond habitats for the stream adapted salamander larvae (discussion chapter 2). In this study the different environmental settings and risks of the relevant phase in the salamanders' life (the larval stage), are compared in detail for the first time. Moreover some potentially relevant selective forces could be identified.

Different effects on salamander larvae growth in ponds versus streams by temperature and food regime

Salamandra salamandra is the only systematically stream reproducing amphibian species in Western Europe (Thiesmeier 2004). First order streams represent open and fairly stable environments in terms of water availability. They are usually perennial and harmfully high temperature regimes could not be shown to occur.

Generally, the most relevant factors influencing growth increment for amphibians are temperature and food availability (Ryan 1986). The salamander larvae growth and developmental time in the streams were most strongly associated with the temperature development as the temperature increases from 10°C during the time of larvipositioning, to more than 12°C at the time of metamorphosis. With the increasing temperature also the growth rates increase, as it would be predicted if the population was not otherwise limited. The growth of stream salamander larvae was as high as the growth shown by larvae raised under unlimited food under respective temperatures in appendix 1. Food in the streams is present in high abundances and the food organism biomass does not change notably during the larval growth season, and it can be concluded that food availability was not a limiting factor for the larval growth in the streams.

The pond environments in contrast are often highly unstable. Oxygen depletion and unpredictably short hydroperiod could lead to mass mortalities and result in a run against time for the developing salamander larvae (Weitere et al. 2004; chapter 1). Furthermore high densities of larvae are confined in the ponds leading to food overexploitation and increased competition (results chapter 1). Weitere et al. (2004) could show previously, that pond-reproducing salamanders show some adaptation

towards reducing the time of larval development and reduced metamorphosis threshold in pond reproducing populations compared to stream populations (see also Table 4.2). The same relationship was also indicated here since pond metamorphs were generally smaller but could appear about one month earlier than stream metamorphs. The metamorphosis size in the pond ecotype was overall highly variable between different individuals but also between the years (see results chapter 2). This was discussed as a result of different life history strategies shaped by the climatic and food variability in the ponds in chapter 2. Especially at the time of larvipositioning the water temperatures in the ponds can sometimes drop below the minimum temperature necessary for growth of 6.47°C (Chapter 2). Nevertheless, it increases quickly and reached 12°C already by mid April. At the time of metamorphosis temperatures of more than 20°C could occur. These higher temperatures in the ponds would allow for a higher growth increment of the salamander larvae during their early development but only if enough food is available. This was nonetheless not the case. While in the early season at high food abundance the growth rates were as high as under unlimited conditions, it quickly decreases with decreasing food availability. It can be assumed that the increasing temperatures even increase the adverse effects, as the increased metabolic demand of the larvae is not sustained by the food availability (Weitere et al. 2004). Pond salamander larvae growth appears thus more limited by the food availability than by the temperature regime.

Salamander larval demand vs. resource availability in ponds and streams

In the pond environments the salamander larval density was distinctly higher in the ponds than in the streams right after larvipositioning. The ponds confine the larvae on a small area and maintain high larval densities throughout the season. This overabundance of predators in comparison to the decreasing food organism biomass resulted in inverted trophic biomass pyramid that further increased the resource limitation in the pond habitats as described in chapter 1 and 3. Due to the low aquatic food availability and the resulting high intraspecific competition for food, the pond larvae acquire alternative substitutes of their diet. We saw that the ingestion of terrestrial arthropods made up a considerable part of their diet, sometimes up to 96 % of all ingested food items (chapter 3). In the stream populations this introduction of subsidies could be shown to be relatively low with a maximum contribution of 8 % to

the ingested food items. It was shown in chapter 3 (Fig. 3.1) that in the ponds terrestrial prey is widely available. Optimal foraging hypothesis (MacArthur and Pianka 1966) would predict the usage of this high quality food resource above the exploitation of low energy planktonic prey. Terrestrial substitutes are mostly taken from the water surface and the pond larvae will spend considerable time floating close to the surface to prey on drowning or ovipositing insects and their eggs (Chapter 1 and 3, also Blaustein et al. 2013).

In the streams where aquatic prey is abundant, the consumption of terrestrial subsidies would not provide a feasible alternative. Floating at the water surface is risky for the salamander larvae because it could lead to increased downstream drift (Thiesmeier and Schuhmacher 1990) and predation by terrestrial predators. Moreover terrestrial substitutes are only available drifting by, unlike in the ponds where they are available on the surface for some time. Thus, it is most likely consumed in the same way as aquatic prey in a sit-and-wait-strategy in the streams and foraging behaviour would not be altered.

Causes of mass mortality in ponds and streams and the effect of reproductive timing

In the ponds, high mortalities could be shown to occur by oxygen depletion, high amounts of decomposition residues, peaks of high temperatures and especially pond drying as demonstrated in chapter 1. These events do occur especially in summer as single unpredictable events but can extinguish the entire larval population of a single pond. Mass mortality events are subjected to a high inter-annual variability but a quick, early development should nonetheless be beneficial to reduce the risk and effects of mass mortality in the ponds.

Anyhow, stream habitats are not devoid of environmental risks. The biggest disruptions of the stream systems occur in spring when heavy rains can result in strong flush floods, increasing mortality in stream fauna by catastrophic drift (Anderson and Lehmkuhl 1963). After ca. 1000 m the first order streams end into wastewater or fish inhabited lower stream segments in which the survival of most headwater organisms is very low. Trout and other larger fish in the upper reaches readily consume salamander larvae. (Thiesmeier 2004). It could be shown that the salamander larvae population density decreases throughout the season as downstream dispersal increases. After strong rain events in mid May no marked larvae could be recaptured at the last release point while

they disperse less than 10 m downstream before. Those larvae that could be recaptured during the later samplings in contrast drifted downstream for more than 100 m and resided in deeper low flowing pools of the streams. Catastrophic drift is majorly connected with this dispersal and usually a result of temporary high discharge of water from the catchment area such as snowmelt or heavy rainfalls. Events of high discharge in headwater streams of temperate climate zones are most common in early spring (Swanson et al. 1998; Lytle 2001; Lytle and Poff 2004).

To avoid some heavy rainfalls, the reproductive timing of the salamanders should be adjusted after early spring flush floods. It could be shown, that salamander larvipositioning in the streams constantly took place on average a month later than in the ponds (see also: Thiesmeier 2004; Weitere et al. 2004) and a connection with avoidance of high flow discharge events after larvipositioning is likely.

Implications for ecologically driven speciation

The colonisation of new habitats is an integral result of dispersal and a key factor for increased genetic diversity in a species (Dieckmann et al. 2004; Kokko and López-Sepulcre 2006). The larval habitat choice of fire salamanders represents an example how flexibility in early life history can increase a species range and genetic variability. Usually species disperse between similar habitats and can increase their range or re-colonise new habitats that are similar to their original habitat. Yet, a high adaptability towards abiotic factors and flexibility in individual developmental patterns can accelerate colonization of new habitat types that are increasingly different from the original (Doebeli and Dieckmann 2003; Sobel et al. 2010). In different habitats different selective pressures can shape the species genotype to match the habitat requirements. In the present study we see that a set of contrasting environmental constraints control the success of fire salamander larvae in the two habitat types. Pond breeders have to rely on the match of temperature and food availability to reach minimum size for metamorphosis before the risk of oxygen depletion, pond overheating or drying increases. Stream salamanders must match their larviposition timing to the probability of flush floods and consequent disturbance regimes. The difference in ecological frameworks might be sufficient to shape the two different genotypes as a first step of "ecological speciation". Thereby it is not geographical isolation, that plays a role in shaping new genotypes but a selection of ecological parameters that act as isolation

barriers in a sympatric population (Rundle and Nosil 2005). In the fire salamanders we might see an example of this ecological isolation during parts of their lifecycle that could provide a powerful mean to initiate micro-evolutionary changes in the population and restrict gene flow.

Conclusion

In this chapter we identified relevant environmental factors that act upon the larval development of fire salamanders that are in the process of a sympatric split into two ecotypes (Steinfartz et al. 2007). The ecological risks and settings lead to conflicting adaptations in terms of life history and growth patterns. Pond ecotype females could be shown to reproduce early in the season to match their offspring with benign abundant food and benign temperature conditions. Their larvae face food depletion and difficult environmental conditions during their late development. Stream reproducing females have to adjust their reproductive timing to the risk of flood events. Their larvae live in an overabundance of aquatic food but their growth is mainly limited by the temperature development in their habitats during their early life stages. Even though the larval phase comprises only a short time in the lifecycle of the salamander, it nevertheless is a crucial step for population success. Multiple factors could be assigned that can result in micro-evolutionary changes in the population driving speciation in the salamanders.

General conclusion

The western fire salamander (*Salamandra salamandra*) displays a very recent colonisation of a new habitat with novel environmental restrictions and selective pressures. In this study it was demonstrated that a new environment influences life history and ecological performance of a single species on the one hand, and ecological functioning of this species on the other. While stream reproducing salamanders live in a perennial system with a predictable temperature regime and abundant food, the pond salamanders must contend with highly instable temperature and limited food conditions, as well as a limited developmental time. Specifically, the variance in rainfall and temperature regime affects the development and behaviour of the larvae in both direct and indirect (as mediated by prey) ways. The high variability of the environmental framework requires not only flexible developmental strategies and the ability of individuals to adjust their timing to the seasonal variation but also necessitates that the entire population adapt to the inter-annual differences. Here it was shown that individual fire salamander larvae could cope with the problems of food shortage by increased ingestion of terrestrial subsidies, as well as the systematic increase in cannibalism. On population level this resulted in different population structuring between the different years, due to the inter-annual variability of matching the essential developmental parameters to the temperature regime and food availability. Nevertheless, individual mortality risk due to anoxic events, or pond drying, remains high in the ponds. Previously the earlier and faster development and lower metamorphosis threshold that has been mainly allotted to the risk of catastrophic events in ponds has been shown (Weitere et al. 2004). Here, the food scarcity in summer could also be shown to be a factor driving the salamander larvae to an early metamorphosis to leave a depleted habitat. The strong priority effects through exploitative competition and cannibalism amongst the larvae additionally explain the earlier reproductive timing of the pond ecotypes. Stream ecotypes in comparison were shown here to be limited by the low temperature regime, and increased mortality generally only occurred after events of flush floods common in early spring. Consequently, stream females showed a later reproduction date than pond females, as evidenced by our work. The contrasting timing of metamorphosis and developmental pattern can be a strong force in aiding the

observed genetic separation of the two ecotypes, which may lead to the forming of different species.

With the adaptation to the novel habitat, the salamander larvae will furthermore also exert massive influence on the communities, and thus ecosystem functioning, in pond habitats. Our current work shows that by depositing larvae in early spring, female fire salamanders import high amounts of larval biomass into the ponds. Due to high mortality rates in the larval phase, and the relatively small size at metamorphosis of the pond-adapted salamanders compared to stream-adapted ones, the biomass export of the metamorphosed salamanders clearly falls below the initial biomass import. Consequently the salamander presence could be shown to directly increase the amount of aquatic-terrestrial subsidy exchange into the aquatic system. Since salamander larval density is initially not bottom-up controlled, but rather a result of the amount of incoming depositing females, high numbers of larvae can persist in the pond habitats. These have a high food demand that could influence food organism community structure. Additionally, the high food demand was also shown to result in indirect effects on the aquatic-terrestrial subsidy exchange, a fact that has been often ignored in the studies of aquatic terrestrial linkage so far. The salamander larvae accelerate the net import of matter into the aquatic habitat, by the feeding on aquatic insect larvae (reducing emerging adults - thus preventing export -) and on terrestrial organisms that fall on the water surface (supporting import). Based on the findings of the present study, previous assumptions on the impact of salamander larvae on macroinvertebrate communities and subsidy exchange were tested experimentally in whole-pond manipulations. In fact it was demonstrated, that most effects on invertebrate biomass and community composition occurred regardless of predator presence, as a result of the abiotic constraints in ephemeral pond systems and prey-specific life cycles. Consequently, the influence of salamander larvae on biomass development biodiversity or food web topology in ephemeral ponds was lower than previously expected. Nonetheless, we could observe a significant direct reduction of a single taxon (chironomids) larval biomass and their emergence. Further, it was confirmed that salamander presence and development in the ephemeral ponds was highly dependent on terrestrial resources that greatly subsidise aquatic food intake. This was shown for the first time to be essential for stabilising the top position in the species-poor food webs of an ephemeral pond.

To conclude, this study shows how one species separates into two ecotypes that complete parts of their development in different ecosystems with individual sets of partly contrasting restrictions. These selective forces are largely responsible for different adaptations in life-history timing and further attributes, which are forceful agents in driving a split between the two ecotypes. Finally, we also describe how integral ecosystem functions, such as aquatic terrestrial subsidy exchange in the new habitats, are influenced by the adaptive traits of a single species. This in turn also sheds light on how the food web and diversity in ephemeral ponds is structured as a result of the abiotic framework, consumptive effects, and terrestrial residuals.

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Appendix - Supplementary Data

In order to quantify the dependence of growth and temperature a total of 154 Larvae were raised to metamorphosis in the laboratory (Weitere 1997, diploma thesis University of Cologne). The larvae were reared at constant temperatures of 8, 10, 11, 15, 20, 21°C with several individuals for each temperature (Table A1). All individuals were housed in groups of same sized individuals in aquaria with a minimum of 2 L water per individual and a water level of 20 cm. All larvae were provided with unlimited supply of food and the water was changed every 5 days. The larvae were weighed weekly until they metamorphosed. For an overview of experimental setups see Table A1).

From the weight increase, we calculated the mean (cohort) growth rate (r, d^{-1}) for all setups during the exponential growth phase by calculating the slope of the linear regression of ln- transformed fresh weight versus time (t, d). Growth rates for all setups were plotted against temperature (Fig. A1). The relationship between growth rate (r) and temperature (T) was linear between 8 and 21°C with

$$r = 4.896\ 10^{-3}T - 0.03127 \qquad (S1)$$

The minimum possible growth temperature (r = 0) can be calculated to be at T = 6.47 °C (Fig. A1). Additionally we calculated the amount of thermal time (in °C d) necessary to reach a theoretical mean metamorphosis weight of 0.84 g (Table A1). The calculated mean thermal time requirement resulted from the time necessary to grow to metamorphosis as measured and the temperature of the setup.

Table A1: Overview over all individual temperature setups.

Individuals per vessel	Initial size [mm]	Temperature [°C]
17	29- 32	10, 20
12	30-37	8, 15, 21
17	28-35	15, 21
10	30-33	15
18	36-43	9, 20

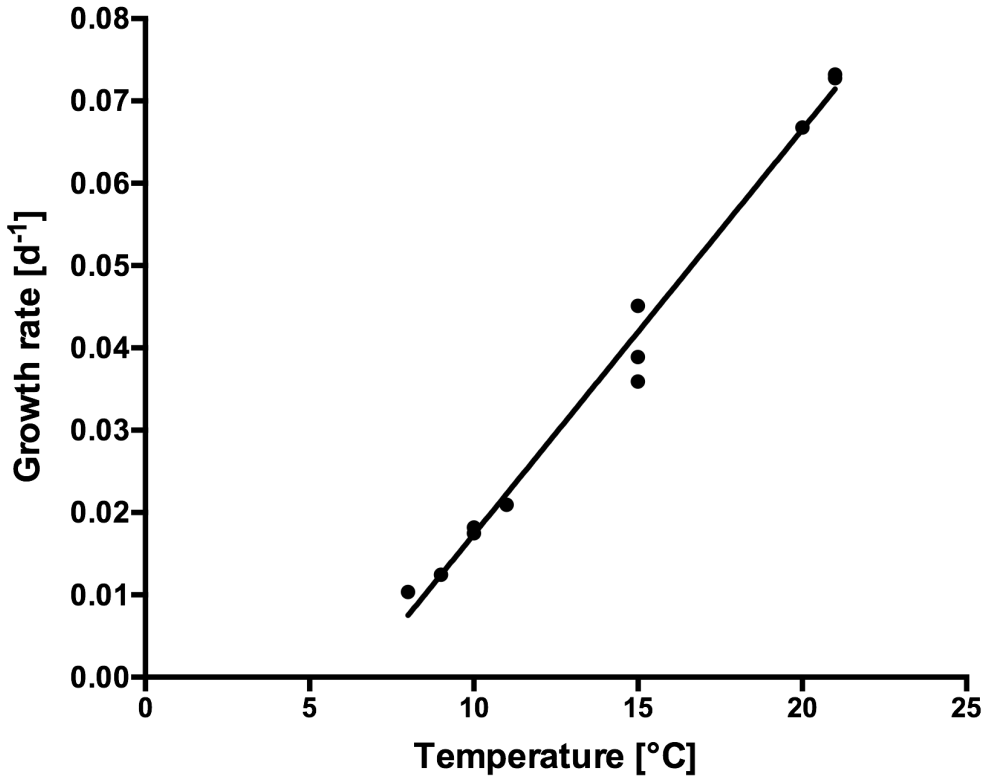

Fig. A1: Mean growth rate (r) versus temperature (°C) under laboratory conditions and unlimited food supply. Linear regression: $r = 4.896\ 10^{-3}\ T - 0.03127$.

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Declaration of contributions

Planning, conduction, evaluation and writing of this study including all manuscripts was accomplishment of **Timm Reinhardt** under supervision and consultancy of Prof. Dr. Markus Weitere (TU Dresden). In two cases further additional data sources were used. (1) Data on salamander larvae and macroinvertebrates as presented in chapter 1. The original data were published in the Diploma thesis of **Markus Weitere** at the University of Cologne in 1997 and Weitere et al. 2004. Those data were used to estimate and evaluate the aquatic terrestrial biomass flux as a part of chapter 1 and in the Appendix (2) All data on macroinvertebrates and salamander phenology in streams gathered by Daniel Geller in a co-investigation used in the Diploma thesis by **Daniel Geller** at the University of Bonn in 2001.
All third person data were recalculated and used in a new context. No data were used in the same way as in other publications. No other than these mentioned data sources were used in the present thesis. All adopted thoughts and data were quoted accordingly. The study was financed by a scholarship of the **Deutsche Bundesstfitung Umwelt** (DBU) from 01.01.2010 to 31.12.2013 issued to Timm Reinhardt at the **Helmholtz-Centre for Environmental Research** - UFZ in Magdeburg.

Chapter 1 of this Thesis was published with minor alterations as:

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Erklärung

Hiermit versichere ich, dass ich die vorliegende Arbeit ohne unzulässige Hilfe Dritter und ohne Benutzung anderer als der angegebenen Hilfsmittel angefertigt habe; die aus fremden Quellen direkt oder indirekt übernommenen Gedanken sind als diese kenntlich gemacht worden. Bei der Auswahl und Auswertung des Materials sowie bei der Herstellung des Manuskriptes habe ich Unterstützungsleistungen von folgenden Personen erhalten:

Professor Doktor **Markus Weitere**, TU-Dresden (Herstellung des Manuskripts und Datenauswertung; Diplom Biologe **Daniel Geller** (Probenname und Auswertung 2011)

..... .

Weitere Personen waren an der geistigen Herstellung der vorliegenden Arbeit nicht beteiligt. Insbesondere habe ich nicht die Hilfe eines oder mehrerer Promotionsberater(s) in Anspruch genommen. Dritte haben von mir weder unmittelbar noch mittelbar geldwerte Leistungen für Arbeiten erhalten, die im Zusammenhang mit dem Inhalt der vorgelegten Dissertation stehen.

Die Arbeit wurde bisher weder im Inland noch im Ausland in gleicher oder ähnlicher Form einer anderen Prüfungsbehörde zum Zwecke der Promotion vorgelegt.

Ich bestätige, dass ich die Promotionsordnung der Fakultät Forst-, Geo- und Hydrowissenschaften der TU Dresden anerkenne

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Ort, Datum, Unterschrift